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# A melanic form of the European grape vine moth, *Lobesia botrana* Den and Schiff (Lepidoptera, Tortricidae), and its genetic basis

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**Summary** — A melanic form of the European grape berry moth, *Lobesia botrana*, is described from a rearing colony maintained at INRA, Bordeaux, France. From the offspring of the crosses with the typical form, $F_1$, $F_2$ and backcrosses, it is established that the melanic form is genetically determined and its inheritance controlled by a single recessive non sex-linked gene *(Roe)*. Likewise, possible practical uses of the genetic marker in field and laboratory tests are discussed.

***Lobesia botrana* / melanic form / recessive gene / inheritance / mutation**

**Résumé — Une forme mélanique de l'eudémis de la vigne, *Lobesia botrana* Den et Schiff (Lepidoptera, Tortricidae), et sa base génétique.** *Nous décrivons une forme mélanique de l'eudémis de la vigne,* Lobesia botrana, *provenant de l'élevage maintenu à l'Inra de Bordeaux, France. À partir de la descendance des croisements avec la forme typique, $F_1$, $F_2$ et rétrocroisements, nous montrons que la forme mélanique est déterminée génétiquement et que l'hérédité est contrôlée par un gène récessif non lié au sexe* (Roe). *En outre, nous discutons des possibilités d'utilisation pratique du marqueur génétique dans des essais au champ et en laboratoire.*

**Lobesia botrana / *forme mélanique / gène récessif / hérédité / mutation***

**Resumen — Una forma mélánica de la polilla del racimo *Lobesia botrana* Den y Schiff (Lepidoptera Tortricidae) y su base genética.** *Se describe una forma melánica de la polilla del racimo,* Lobesia botrana, *proveniente de la cría que se mantiene en el INRA de Burdeos, Francia. A partir de la descendencia de los cruzamientos con la forma típica, $F_1$, $F_2$ y retrocruzamientos, se establece que la forma melánica está determinada genéticamente y su herencia controlada por un gen recesivo no ligado al sexo* (Roe). *Asimismo, se discuten las posibles aplicaciones prácticas del marcador genético en experiencias de campo y laboratorio.*

**Lobesia botrana / *forma melánica / gen recesivo / herencia / mutación***

## INTRODUCTION

The grape vine moth *Lobesia botrana* Den and Schiff is known as the most important grape pest in Europe, North Africa and West Asia, specially in southern palaeartic vine-growing areas (Bovey, 1966; Roehrich and Boller, 1991). Therefore, owing to viticultural importance in the

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Bordeaux area, a rearing colony has been maintained for over 100 generations in INRA (Stockel et al, 1989) for scientific purposes.

In May 1993, a few adults darker than typical individuals were found in the colony, showing a classic melanic colour pattern (sensu Betz, 1962). Because of the potential applied interest of this finding, these specimens were reared apart and multiplied isolately. Melanic pattern was only expressed in adults.

A comparison with Bovey adult description (1966) and with reared and field-sampled imagines, showed a marked difference between the melanic and the typical specimens of *L botrana*. Thus, in this paper the melanic form is described and its genetic basis is studied.

## DESCRIPTION OF THE MELANIC FORM OF *LOBESIA BOTRANA*

### *Male*

'France, Bordeaux, May-1993, Torres-Vila *leg*'. Wingspan 11.5 mm. Head: deep brownish, *Labial palpus* with distally brown and basally light brown individual scales, altogether brownish. *Antennae* with the scape and *flagellum* concolorous with head, and single antennal segments distally black annulated. Eyes brown. Thorax: deep brown with checkered scales like the *palpus*. *Tegulae* equally pigmented. Dorsal crest brown ferroginous. Forewings: ground colour bluish gray. Fasciae brown ferroginous concolorous with thoracic dorsal crest scales. A black preapical spot. Median fascia including little black imbricated spots. Moreover, dusted black scales disorderly scattered. Scales line along the *costa* and dorsum darker than in wing ground. Cilia grayish brown with a paler apical tip, and a cream basal line along the *termen* reaching the *apex* and *tornus*. Underside deep gray metallic, gradually darker towards the *costa* and *apex*. Hindwings: light gray with argenteous hue, darker towards the *apex*. *Cilia* and cubital tuft grayish, with a paler basal line. Underside uniform light gray argenteous. Abdomen: altogether deep brown, hair pencils included. Legs: deep brown, epiphysis and spurs included, with golden gleams. See figure 1.

### *Female*

'France, Bordeaux, May-1933, Torres-Vila *leg*'. Wingspan 12.5 mm. As a whole, coloration pattern as the male. The abdomen ventrally concolorous with the abdominal sclerotized sternite VIII. See figure 1.

### *Variation*

7 ♂♂ and 11 ♀♀ were examined. Size and hue of melanic form forewings *fasciae* are more or less variable among specimens, just like size and distribution of black spots.

### *Differential diagnosis*

Adults of the melanic form differed from typical specimens, as a whole, in the melanic pattern described. In particular, melanic form forewings *fasciae* are not shaped by a pale cream border. The legs are completely deep brown, without alternated pale cream and brown bands characteristic of the typical form. The abdomen is deep brown in the melanic and cream in the typical form; this is the differential character most evident macroscopically. The typical grayish blue preapical spot is diluted on bluish gray ground colour of melanic forewing. The black drawing on typical thorax vanishes on deep brown coloration of melanic thorax.

## GENETIC BASIS OF THE MELANIC FORM

### *Material and methods*

#### Insects

Typical and melanic adults used in crosses were obtained from our colony. The melanic individuals were taken from a pure strain reared apart from the main typical population. Environmental conditions in rearing were: 22 ± 1 °C, 60 ± 10% RH, photoperiod 16:8 (light:dark) and a 500 lux light intensity during the photophase. Pupae were isolated, to insure virginity of test moths, in glass tubes (70 x 9 mm Ø) stoppered with cardboard plugs. Emerged moths were collected daily and sexed for experimental crosses. For more details about the rearing method see Stockel et al (1989).

## Experimental device and method

A device for mating, ovopositing, and rearing larvae simultaneously was prepared with a 300 mL cylindrical cage made of transparent plastic. A 60 mL volume of liquid synthetic medium was poured into the cage and, after agar solidification, the surface was striated to facilitate larval installation and feeding. The cage was closed with muslin held in position by a rubber band. With this operating method, the whole offspring can be recovered.

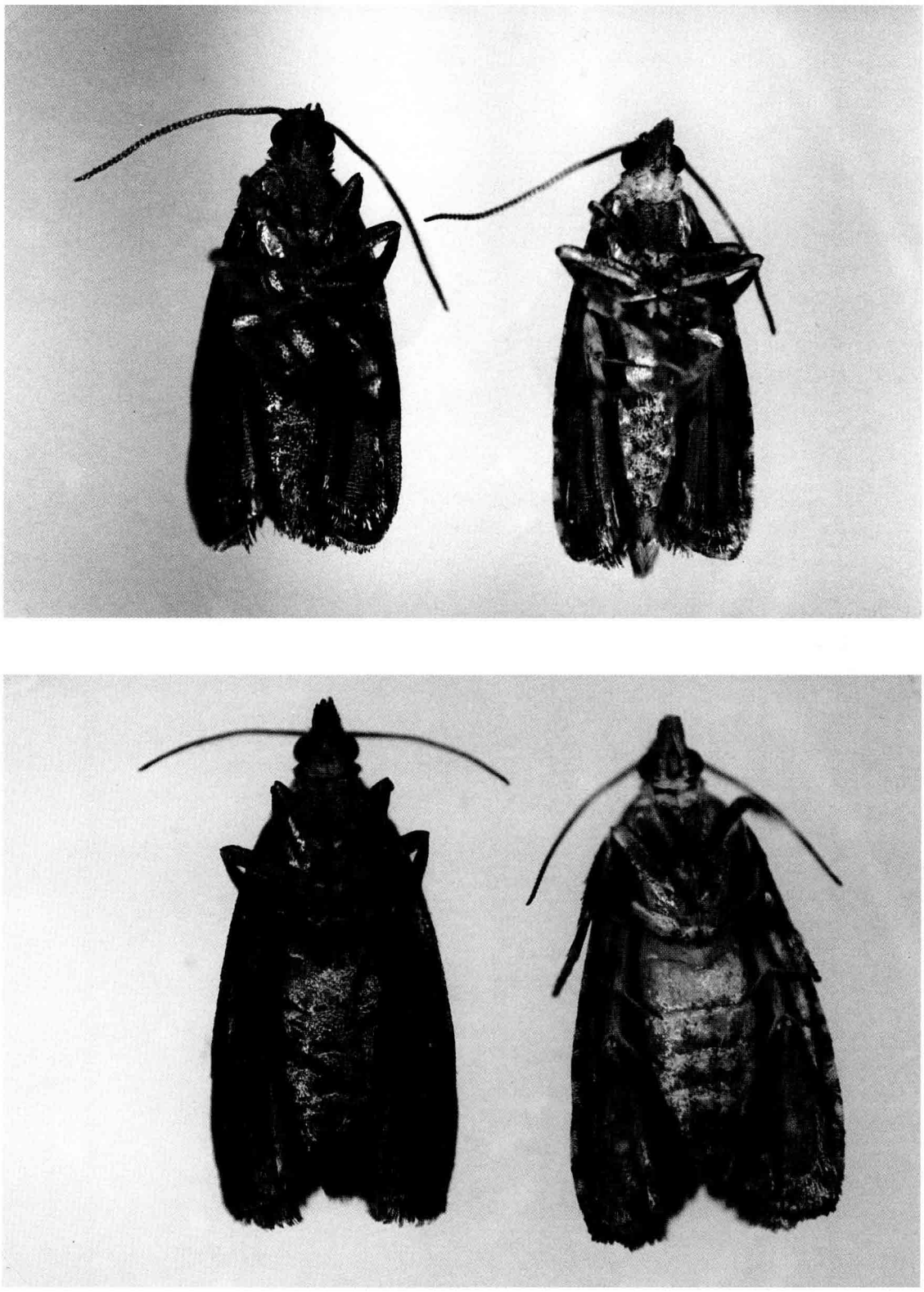

**Fig 1.** Melanic (on the left) and typical (on the right) males (at the top) and females (at the bottom) of *Lobesia botrana* (photos G Attard).

**Table I.** Segregations obtained from the $F_1$, $F_2$ and backcrosses between typical and melanic phenotypes of the European grape vine moth, *Lobesia botrana*.

| | *Parents* | | | *Offspring* | | | | | | *Typical:melanic* | | $\chi^{2}$ [b] | p *(%)* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Sex* | | *Replicates* | *Typical* | | | *Melanic* | | | *segregation* | | | |
| | ♀ | ♂ | *n* | ♀ | ♂ | *Total* | ♀ | ♂ | *Total* | *Obtained* | *Theoretical* [a] | | |
| Crosses | | | | | | | | | | | | | |
| $F_1$I | Melanic | Typical | 9 | 145 | 149 | 294 | – | – | – | 1:0 | 1:0 | – | – |
| $F_1$II | Typical | Melanic | 11 | 212 | 220 | 432 | – | – | – | 1:0 | 1:0 | – | – |
| Total | | | 20 | 357 | 369 | 726 | – | – | – | 1:0 | 1:0 | – | – |
| F2I | $F_1$I | $F_1$I | 12 | 74 | 80 | 154 | 24 | 18 | 42 | 3.66:1 | 3:1 | 1.15 | 31.9 ns |
| F2II | $F_1$II | $F_1$II | 11 | 65 | 55 | 120 | 15 | 15 | 30 | 4.00:1 | 3:1 | 1.74 | 15.3 ns |
| Total | | | 23 | 139 | 135 | 274 | 39 | 33 | 72 | 3.81:1 | 3:1 | 3.24 | 7.9 ns |
| Backcrosses | | | | | | | | | | | | | |
| BI | $F_1$I | Melanic | 5 | 9 | 18 | 27 | 11 | 13 | 24 | 1.13:1 | 1:1 | 0.08 | 100.0 ns |
| BII | Melanic | $F_1$I | 5 | 17 | 15 | 32 | 14 | 15 | 29 | 1.10:1 | 1:1 | 0.07 | 100.0 ns |
| BIII | $F_1$II | Melanic | 4 | 7 | 10 | 17 | 11 | 14 | 25 | 0.68:1 | 1:1 | 1.17 | 31.9 ns |
| BIV | Melanic | $F_1$II | 5 | 35 | 28 | 63 | 29 | 28 | 57 | 1.11:1 | 1:1 | 0.21 | 100.0 ns |
| Total | | | 19 | 68 | 71 | 139 | 65 | 70 | 135 | 1.03:1 | 1:1 | 0.06 | 100.0 ns |

[a] In agreement with the recessive single no sex-linked gene hypothesis of melanic form; [b] $\chi^{2}$ with continuity correction factor when $n < 200$.

Only one 24-h-old moth pair was placed in each cage. Water was provided by a wet cotton wool wick. After mating, the female normally ovoposited on the cage wall because it requires no natural oviposition substrate (Torres-Vila, 1995). At death, the adults were removed. Newly emerged larvae descended the cage and reached the medium to feed on. When larvae reached the last instar, some strips of corrugated straw paper were added for pupation. Pupae were collected and isolated in vials like those used in rearing. Emerged adults from the different crosses were inspected, and typical and melanic phenotypes recorded.

### Crosses

To explain the genetic control of the melanic form the following crosses were carried out (T: apical form and M: melanic form): ♀M x ♂T (F1I) and the reciprocal ♀T x ♂M (F1II), self-fertilization of F1I and F1II (F2I and F2II respectively) and all the possible backcrosses (BI: ♀F1I x ♂M, BII: ♀M x ♂F1I, BIII: ♀F1II x ♂M and BIV: ♀M x ♂F1II). Each cross (F1I, F1II, F2I and F2II) was replicated twelve times and each backcross (BI, BII, BIII and BIV) six times.

### Statistical analysis

The agreement between observed and theoretical segregations was checked by a $\chi^2$ test, with a continuity correction factor when the analyzed sample was less than $n = 200$, using the package STATITCF (ITCF, 1988).

## RESULTS

The segregations obtained in the crosses are summarized in table I. In $F_1$I, of twelve pairs, two left no offspring and in another one, a segregation of 22:12 typical: melanic was obtained, attributable to male heterozygosis (see the *Discussion).* Because of that, all the adults of this replicate were eliminated and were not used as $F_2$I parents. From the nine remaining pairs, 294 typical adults (145♀ + 149♂) were obtained. In $F_1$II, of twelve pairs, one left no offspring. From the eleven remaining pairs, 432 typical adults (212♀ + 220♂) were obtained. In $F_2$I, the twelve pairs tested left typical and melanic offspring, except one case with only typical phenotype due to a limited progeny (three adults). In total, 154 typical adults (74♀ + 80♂) and 42 melanic adults (24♀ + 18♂) were obtained. In $F_2$II, of twelve pairs, one left no offspring. In the offspring from the eleven remaining pairs melanic phenotype was observed. In total, 120 typical adults (65♀ + 55♂) and 30 melanic adults (15♀ + 15♂) were obtained. In the backcrosses, of the 24 pairs altogether tested, five pairs left no offspring (see table I for each particular backcross). In the offsprings from the 19 remaining pairs, both melanic and typical forms were obtained, except for two cases with only melanic offspring, certainly due to a limited progeny (one and two imagines respectively).

## DISCUSSION

The absence of segregation in the melanic population from the isolated rearing (20 generations at this time) indicates that the character is fixed in a pure strain. Moreover, the biotic potential of melanic and typical strains is similar.

As regards the genetic basis, the absence of melanic individuals in both $F_1$ indicates dominance of typical form over the melanic one. Furthermore, segregations obtained in both $F_2$ are in agreement with that expected (3:1, typical:melanic) for a recessive monogenic inheritance. Moreover, the existence of melanic females per se points out that the character is not W-linked (hologynic). Remember that sexual determination in Tortricidae is of Abraxas type, with the female sex heterogametic, ♀ZW and ♂ZZ. The absence of segregation in $F_1$II and the presence of melanic males in $F_2$I also excludes that the character is Z-linked. From both $F_2$ data, a partially sex-linked inheritance is not evident either. To sum up, the segregations obtained indicate that the melanic form is not sex-linked. These results are corroborated by the segregations obtained from the four backcrosses, which are not significantly different from the expected one (1:1, typical:melanic).

In conclusion, the segregation patterns obtained from the experimental crosses clearly support that the melanic form is genetically determined and regulated by a recessive single gene (*Roe* melanic gene is named *Roe* in honour of Prof Dr R Roehrich, for his scientific accomplishments in Tortricidae and particularly in *L botrana*) that is not sex-linked.

However, in relation to the colour pattern of the forewings particularly, the inheritance could be rather more complicated, with intermediate dominance or modifier gene(s) involved. In fact, the colour pattern of the forewings in some $F_1$ adults (classified as typical) tends to be slightly darker than in the typical phenotype, but this trait was not evaluated in the present study. In other cases of melanic inheritance under general

monofactorial control in Lepidoptera, an intermediate forewing colour in heterozygous individuals has also been observed (see Ford, 1965).

With regard to the segregation obtained from the single pair signaled in $F_1$I (see *Results),* where the melanic phenotype appears, it must be remembered that the *Roe* gene is present in the typical strain. In assessing the frequency of the melanic form in our typical colony, from a sample of 412 individuals examined, three were melanic (0.72%). Thus, assuming the panmictic condition in the laboratory population, the proportion of heterozygous individuals in the colony is estimated about 15.5%, following the Hardy–Weinberg law. The disagreement observed between this estimated percent and the one obtained in $F_1$ (1/21 $\approx$ 5% of heterozygous individuals) is certainly due to the fact that the most typical individuals (homozygous) were chosen as $F_1$ parents and, as result of that, the frequency of heterozygous was underestimated.

The occurrence of melanic forms is relatively usual in laboratory rearings, due to the mutation likelihood and to the higher consanguinity and homozygosity (see Poitout, 1973). Thus, several melanic or other chromatic forms with inheritance controlled by a single recessive gene have been described from Lepidoptera rearings, for example in *Laspeyresia pomonella* L (Charmillot and Rosset, 1977), *Spodoptera exigua* Hb (Poitout, 1973) and *Agrotis segetum* Den and Schiff (Monastyrskii, 1990), and also probably in *Pieris rapae* L (Tatchell and Riley, 1989) and *Mamestra brassicae* L (R Bues, S Poitout, personal communication). Unusually, the melanism in laboratory rearings has not the genetic basis described, as occurs in the population of *Trichoplusia ni* Hb studied by Toba et al (1970), where the character is regulated by a single dominant gene, lethal when homozygous. However, in natural conditions, in Lepidoptera species with industrial melanism, including the peppered moth *Biston betularia* L, the implicated genes are usually dominant (Ford, 1965).

As regards the practical uses, the availability of a macroscopic genetic marker in *L botrana* has a great interest, including in-field research on the dispersal and population estimates of larvae and adults with release–capture techniques (to recognize larval phenotype, rearing is necessary for reaching the adult stage) and laboratory competition assays. Through natural marking, it is possible to avoid the undesirable effects of artificial marking procedures, like anesthesia, additional manipulations and internal or external markers themselves (paints, dyes, radiations, labels, mutilations and others). Thus, genetic marking has been often applied in some Lepidoptera species (Hutt and White, 1975; Audemard, 1980; Bartlett and Lingren, 1984).

On the other hand, it is necessary to check previously that melanic form fitness is not reduced and that the *Roe* gene is not linked with other detrimental or deleterious genes. For example, under laboratory conditions, the melanic moths of *S exigua* have a lower biological value than the normal ones (Poitout, 1973). Also, remember that natural selection under field conditions may operate differentially (Ford, 1965; Southwood, 1966), as has been particularly shown in another tortricid, *L pomonella* (Hutt and White, 1975). In this species, the golden strain employed was comparable to the gray (normal) one in mating activity in a laboratory test, but not in release–capture studies in the field, where released gold males were less recaptured.

The melanic form regulated by the *Roe* gene is the first colour form described in *L botrana.* However, in other tortricid species a number of melanic forms with inheritance under monofactorial control are known (see Benz, 1991). In the more extensively studied *L pomonella,* a number of melanic forms under recessive monogenic control have been reported, including brown (= *f putaniana* Stgr) (Rice, 1941) and *grey* (Charmillot and Rosset, 1977), and another unnamed one (R Bues, S Poitout, personal communication).

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