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## THE *MITOURA SPINETORUM* COMPLEX IN NEW MEXICO AND THE VALIDITY OF *M. MILLERORUM* (LYCAENIDAE: THECLINAE)

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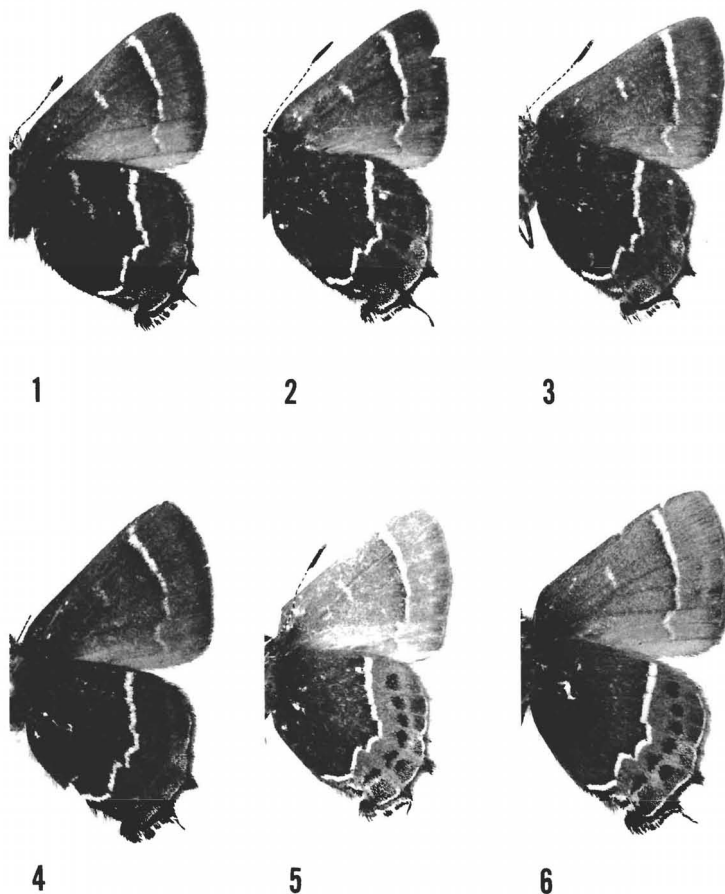
**ABSTRACT.** *Mitoura millerorum* (Clench) has been considered either a synonym of *M. spinetorum* (Hewitson) or a distinct species that does or does not occur in New Mexico. In a sample of 128 individuals collected in the vicinity of Weed, New Mexico, wing pattern characters previously proposed to distinguish *M. spinetorum* from *M. millerorum* were uncorrelated. Likewise, proposed genitalic differences represented a small portion of continuous unimodal variation. The wing pattern and genitalia of the types of *M. spinetorum* and *M. millerorum* fall within this range of variation. There is no published evidence supporting the hypothesis that *M. millerorum* is a distinct species.

**Additional key words:** genitalia, wing pattern, continuous genitalic variation, taxonomy.

Specific identity of a female in the *Mitoura spinetorum* (Hewitson) complex from the vicinity of Weed, Otero County, New Mexico, has engendered controversy. There are three published hypotheses concerning its identity, and, more generally, concerning the validity of *M. millerorum* Clench as a distinct species.

Clench (1981) collected the female from Weed and considered it an aberration of *M. spinetorum*. In that paper, he distinguished the Mexican *M. millerorum* from *M. spinetorum* by (1) presence of a ventral hindwing basal line (Fig. 1), lacking in *M. spinetorum* (Fig. 6); and (2) ventral hindwing postmedian line nearly straight from veins Cu2 to 2A (Fig. 1), with a "tooth" in *M. spinetorum* (Fig. 6). He also outlined statistical differences. Although the Weed female has the basal line of *M. millerorum*, it otherwise agrees with *M. spinetorum*.

Johnson (1985) proposed that the Weed female is *M. millerorum*, a new distribution record for the United States. He considered presence of a ventral hindwing basal line "the best overall superficial character for recognizing *millerorum*" and listed seven genitalic differences be-



FIGS. 1-3. Ventral wing surfaces with a hindwing basal line that is well-developed (Fig. 1), faint (Fig. 2), or reduced to a few scales (Fig. 3). Angle of postmedian line between veins Cu2 and 2A is  $105.8^\circ$  (Fig. 1),  $87.1^\circ$  (Fig. 2),  $92.9^\circ$  (Fig. 3).

FIGS. 4-6. Ventral wing surfaces without a hindwing basal line showing variation in angle of postmedian line between veins Cu2 and 2A:  $105.3^\circ$  (Fig. 4),  $94.2^\circ$  (Fig. 5),  $82.1^\circ$  (Fig. 6).

tween *M. spinetorum* and *M. millerorum*. He concluded that shape of the cubital postmedian line did not distinguish the two species.

Scott (1986) treated *M. spinetorum* and *M. millerorum* as conspecific. He noted that specimens from the Sacramento Mountains of New Mex-

ico, such as the Weed area, occasionally have traces of a basal line on the ventral hindwing. Presumably, he considered these populations to be geographically and phenotypically intermediate between *M. spinetorum* and *M. millerorum*. However, he presented no evidence refuting the hypothesis that these species are distinct and sympatric in the Sacramento Mountains.

The purposes of this paper are to determine whether there are one or two species in the *M. spinetorum* complex in the vicinity of Weed, New Mexico, and to determine whether *M. millerorum* is a distinct species. I quantify Clench's ventral hindwing postmedian line character and Johnson's genitalic characters using 128 specimens (some with and some without a ventral hindwing basal line) collected near Weed. If there are two sympatric species distinguished by these characters, then they should have bimodal distributions correlated with the presence or absence of a ventral hindwing basal line. Finally, I compare the types of *M. spinetorum* and *M. millerorum* with variation at this site.

#### MATERIALS

Steve Cary, Dick Holland, and I collected 44 males and 84 females in the *M. spinetorum* complex on Forest Road 164, 1–4.5 miles from its intersection with State Road 130, in Lincoln National Forest along the Rio Penasco, Otero County, New Mexico, on 16, 21, and 22 June 1986. Clench's Weed female was caught on 12 June 1960 along the Rio Penasco and, if not at the same site, then within two miles of it. Specimens and their genitalia were deposited in the National Museum of Natural History.

#### METHODS

Distances were measured using a binocular microscope with a drawing tube and a digitizing pad with a puck. To quantify angles, I measured distances among the vertex and one point on each line forming the angle (equivalent to measuring the sides of a triangle) and calculated the angle using the Law of Cosines (Protter & Morrey 1965). To estimate measurement precision, I re-measured each character in 20% of the specimens.

I graphed results and tested them statistically, presenting means, standard deviations, ranges, and medians. Measurements were illustrated as histograms about the mean with quantiles one standard deviation wide. Because the distribution of measured ratios and angles is often skewed, I tested differences between specimens with and without a ventral hindwing basal line using the non-parametric Wilcoxon two-sample test corrected for ties (Sokal & Rohlf 1969).

I quantified shape of the male saccus by assigning a rank to each

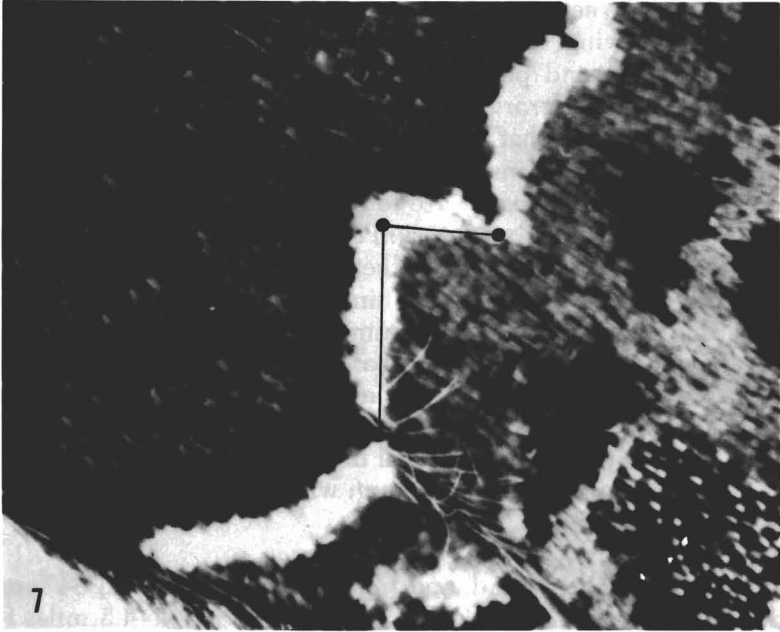


FIG. 7. Points specifying angle formed by the ventral hindwing postmedian line between veins Cu2 and 2A.

specimen. George Venable used the Transform command of Adobe Illustrator '88 software to produce an equally graded series of six shapes that spanned the range of variation in saccus width. Elaine Hodges then assigned each male to a rank by determining to which of the six figures its saccus was most similar. Venable and Hodges are professional entomological illustrators at the Smithsonian Institution and knew nothing about the project except that I was quantifying shape variation. I graphed results by rank and tested differences between specimens with and without a hindwing basal line as described above.

## RESULTS

### Ventral Hindwing Basal Line

A ventral hindwing basal line is the most important wing pattern character state distinguishing *M. millerorum* from *M. spinetorum* (Clench 1981, Johnson 1985). The line is fuscous, bordered basally with white scales, and not as conspicuous as the postmedian line (Clench 1981). Because specimens in the Weed sample varied from fresh to worn and because expression of the line varied (Figs. 1–3), I examined each individual with a binocular microscope at 24 power to score pres-

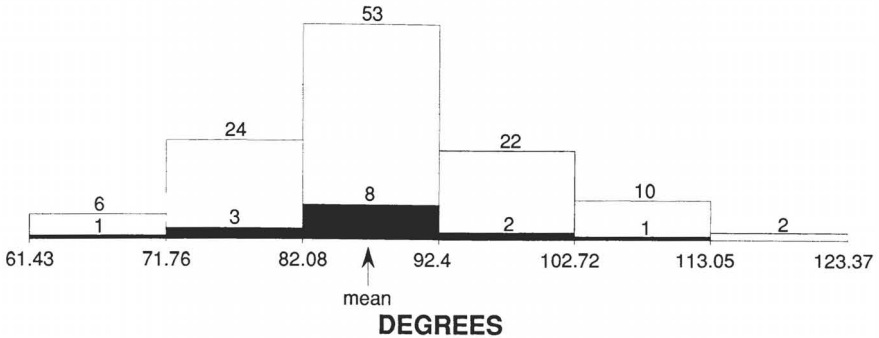


FIG. 8. Distribution of the angle formed by the ventral hindwing postmedian line between veins Cu2 and 2A. White columns represent the complete sample ( $n = 117$ ), whereas black columns are a subsample of specimens with a basal line ( $n = 15$ ).

ence or absence. Two specimens had the white portion of the line reduced to 2–3 scales, but in most cases presence was easily scored without a microscope.

Fifteen of 128 specimens (11.7%) had a ventral hindwing basal line (Figs. 1–6). It was present in 2 of 44 males (4.5%) and 13 of 84 females (15.5%). At the study site, either *M. spinetorum* is dimorphic for the ventral hindwing basal line or it is sympatric and synchronic with *M. millerorum*. The original Weed female was not an aberration.

#### Ventral Hindwing Postmedian Line

The “W” in the ventral hindwing postmedian line of *M. spinetorum* is not recognizable in *M. millerorum* because the line is nearly straight from vein Cu2 to 2A (Clench 1981). I quantified the angle formed by the postmedian line between veins Cu2 and 2A. The three points specifying the angle were: (1) intersection of the postmedian line with vein Cu2; (2) basal-most point of the postmedian line in the cell (vertex); and (3) intersection of the postmedian line with vein 2A (Fig. 7). All were scored in the middle of the postmedian line.

I graphed distribution of postmedian line angles (Fig. 8) and illustrated variation (Figs. 1–6). Eleven specimens were omitted because their hindwings were torn or too worn to be measured. The mean angle was  $87.2^\circ$  ( $s = 10.32^\circ$ ), ranging from  $62.1^\circ$  to  $118.8^\circ$ , and the median was  $85.3^\circ$ . Second measurements of 23 specimens differed from the first measurements by an average of  $4.2^\circ$  ( $s = 3.65^\circ$ ). I also graphed the distribution of angles for the 15 specimen subset that had a ventral hindwing basal line (Fig. 8).

The distribution of postmedian line angles was unimodal (Fig. 8), and this angle was not statistically greater in specimens with a basal

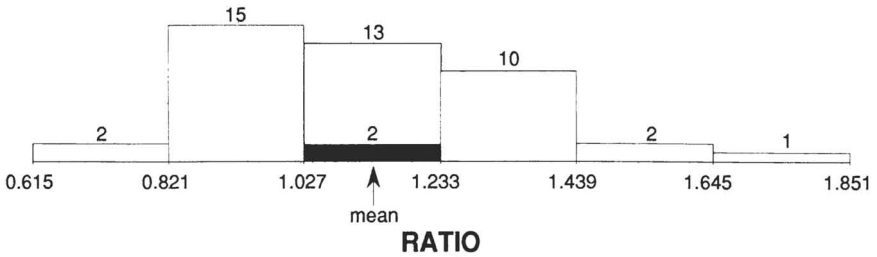
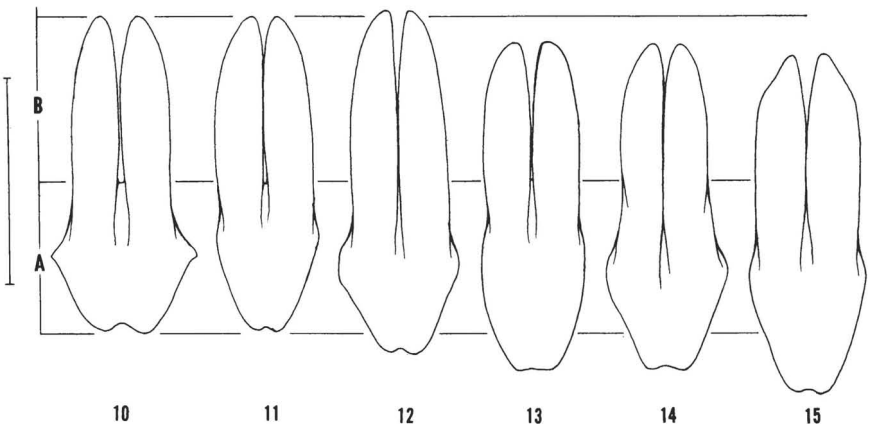


FIG. 9. Ratio of basal to distal length of male genitalia valves. White columns represent the complete sample ( $n = 43$ ), whereas the black column is a subsample of males with a basal line ( $n = 2$ ).

line on the ventral hindwing ( $t_s = 1.167$ ). Of those with the basal line, seven had angles above the median and eight below.

### Male Genitalia

**Valves.** The first male genitalic character that Johnson (1985:120) used to distinguish *M. millerorum* from *M. spinetorum* was "bilobed area markedly larger as contrasted to caudal length of valvae." Johnson (1981) figured the "bilobed configuration" as the anterior part of the valves and the "caudal extension" as the posterior part, but did not specify where each begins or ends. I thus measured valve length along the sagittal plane in ventral aspect from the anterior edge of the valves to the point where they separate and then from this point to their distal



FIGS. 10-15. Variation in male genitalia valves (ventral aspect) of specimens without a ventral hindwing basal line. The central horizontal line intersects the point at which the valves are joined medially. The ratio of valve lengths for the specimen in Fig. 10 is distance A divided by distance B. Scale 1 mm.

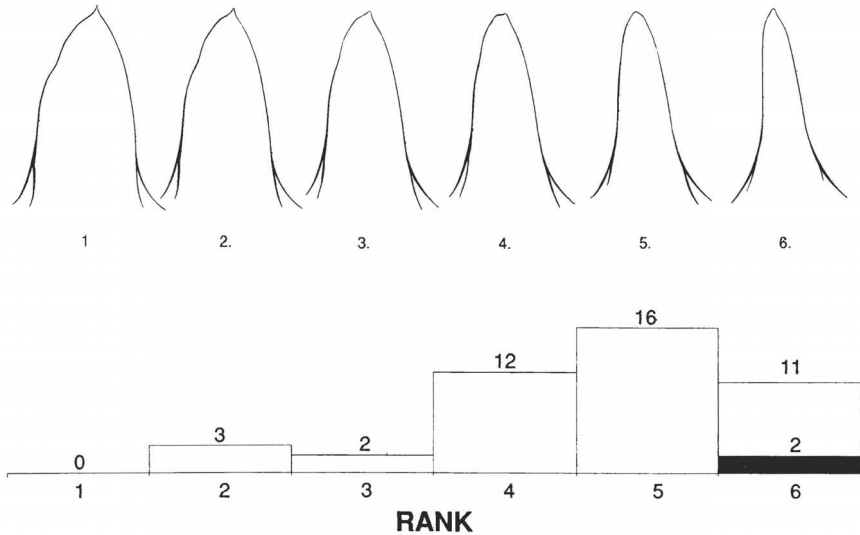


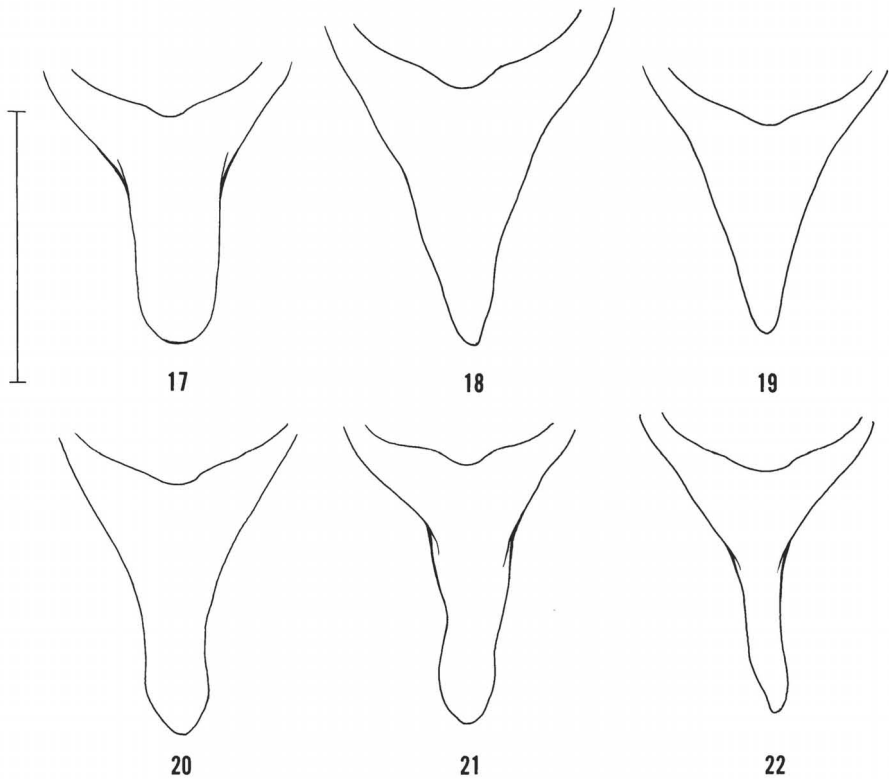
FIG. 16. Model ranks for male genitalia saccus and number of specimens placed in each rank. White columns represent the complete male sample ( $n = 44$ ), whereas the black column is a subsample of males with a basal line ( $n = 2$ ).

tips (Fig. 10) and calculated the ratio of basal to distal lengths. It was not possible to compare absolute lengths because Johnson's illustrations omitted a scale line.

I graphed distribution of valve length ratios for 43 specimens (Fig. 9)—one specimen's valves were damaged during preparation. The mean ratio was 1.13 ( $s = 0.206$ ), ranging from 0.82 to 1.72, and the median was 1.10. Repeated measurements on 9 specimens differed by an average 0.07 ( $s = 0.061$ ). Johnson's (1985) illustration of *M. millerorum* had a ratio of 1.19 and that of *M. spinetorum* was 1.07.

The distribution of valve length ratios was unimodal (Fig. 9) and did not differ significantly between specimens with and without a basal line ( $t_s = 0.231$ ). Males with the line had ratios (1.09, 1.11) just above and below the median. I illustrated valves because length ratios measured only one aspect of variation (Figs. 10–15). Width and length of both the basal and distal parts varied, and the differences between *M. spinetorum* and *M. millerorum* that Johnson illustrated represented a small portion of this variation.

**Saccus.** Johnson's (1985:120) second distinctive male genitalic character of *M. millerorum* was "saccus widely parabolic" in contrast to the "parabolic" saccus of *M. spinetorum* (Johnson 1976). Although he did not show that saccus shape was parabolic, Johnson (1976:8) defined "parabolic" as "saccus gradually tapering and rounded cephalad" and



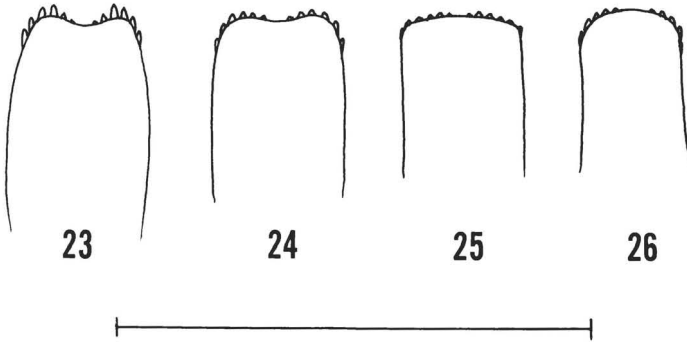
FIGS. 17–22. Variation in shape of male genitalia saccus (ventral aspect) in specimens without a basal line on the ventral hindwing. These specimens were placed in rank 2 (Fig. 17), rank 3 (Fig. 18), rank 4 (Fig. 19), rank 5 (Figs. 20, 21), rank 6 (Fig. 22). Scale 1 mm.

“wide-parabolic” as “saccus parabolic, but tapered less gradually.” Thus, male *M. spinetorum* should have a less tapered saccus, as in Johnson’s (1985) illustrations.

I graphed the number of specimens that were ranked in each of six graduated saccus shapes (Fig. 16). Mean rank was 4.7 ( $s = 1.12$ ), ranging from rank 2 to rank 6, and the median was rank 5. Johnson’s illustration of *M. spinetorum* was placed in rank 3 and that of *M. millerorum* in rank 2.

The distribution of saccus shape ranks was unimodal (Fig. 16) and there was no statistically significant difference ( $t_s = 1.747$ ) between individuals with and without a hindwing basal line. Two males with a line were placed in rank 6, but Johnson’s illustration of *M. millerorum* was placed in rank 2.





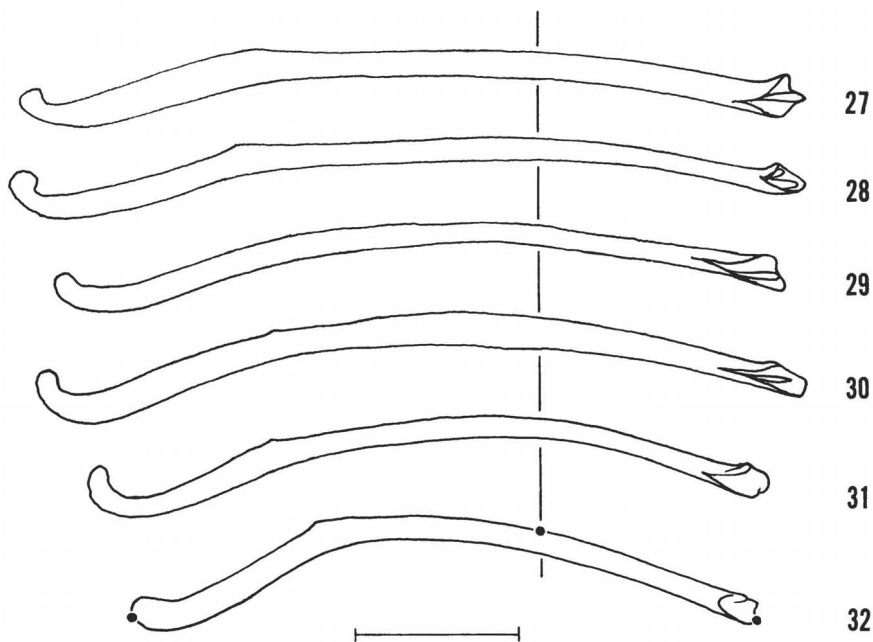
FIGS. 23–26. Variation in shape of the tip of the dorsal cornutus, which was scored as “bifurcate” (Figs. 23, 24) or “not bifurcate” (Figs. 25, 26). Scale 1 mm.

Quantification does not reflect the complexity of saccus shape variability (Figs. 17–22). The saccus can be rounded or triangular (Figs. 17, 18), wide or narrow (Figs. 17, 22), and asymmetrical to the left or right (Figs. 21, 22). Differences in saccus shape between *M. spinetorum* and *M. millerorum*, as illustrated by Johnson, represent a very small portion of intraspecific variability.

**Dorsal cornutus.** Johnson’s (1985:120) third distinctive male genitalic character of *M. millerorum* was “cephalad cornutus at aedeagal terminus bifurcate.” The posterior end of the dorsal cornutus is curved upwards a variable amount, is usually twisted, and has teeth on the posterior edge that are sometimes absent or reduced in size in the middle. I oriented the genitalia so that the cornutus tip was at a right angle to the plane of view. If the posterior edge was indented (viewed at 125 power), it was scored as “bifurcate” whereas if it was straight or convex, it was scored as “not bifurcate.” Of nine specimens scored a second time, one differed from the original assessment.

Shape of the dorsal cornutus did not distinguish *M. millerorum* from *M. spinetorum*. Twenty of 41 specimens (49%) without a ventral hindwing basal line (*M. spinetorum* phenotype) had a “bifurcate” cornutus as did one of two specimens with a ventral hindwing basal line (*M. millerorum* phenotype). Shape of the posterior edge appeared to vary continuously. I illustrated (Figs. 23–26) examples representing the range of variation in the sample.

**Penis.** Johnson’s (1985:120) fourth male genitalic character for distinguishing *M. millerorum* was “caudal one-third of aedeagus distinctly curved (60°) in known specimen.” He did not specify the number of degrees that the penis of *M. spinetorum* curves, but I infer that it is less curved than that of *M. millerorum*.



FIGS. 27-32. Variation in curvature and length of the penis (lateral aspect) of specimens without a ventral hindwing basal line. The vertical line intersects the point one-third of the distance from distal end of each penis. The three points in Fig. 32 specify the angle of curvature that I measured; the middle point was the vertex. Scale 1 mm.

I calculated penis curvature (Figs. 27-32) by measuring the angle specified by (1) the most anterior point of the penis, (2) the ventral tip of the penis, and (3) the penis dorsal surface one-third of the distance from the posterior tip (vertex) (Fig. 32). I used an ocular scale with a perpendicular line to determine the last point.

I graphed distribution of penis curvature for 43 specimens (Fig. 33) and illustrated variation (Figs. 27-32). The mean angle was  $158.6^\circ$  ( $s = 5.54$ ), ranging from  $148.9^\circ$  to  $168.5^\circ$ , and the median angle was  $157.9^\circ$ . Repeated measurements on 9 specimens differed by an average  $3.9^\circ$  ( $s = 3.41$ ).

The distribution of angles was slightly bimodal (Fig. 33), but penis curvature of individuals with a ventral hindwing basal line was not significantly different from that of other specimens ( $t_s = 1.211$ ). One male with a line had an angle of  $158.6^\circ$ , the mean for the entire sample, while the other had an angle of  $165.9^\circ$ , a result contrary to the hypothesis that the penis of *M. millerorum* curves more sharply than that of *M. spinetorum*.

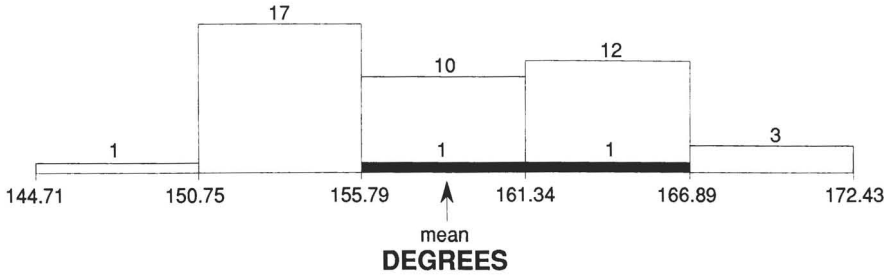


FIG. 33. Penis curvature. White columns represent the complete sample ( $n = 43$ ), whereas the black column is the subsample of males with a basal line ( $n = 2$ ).

### Female Genitalia

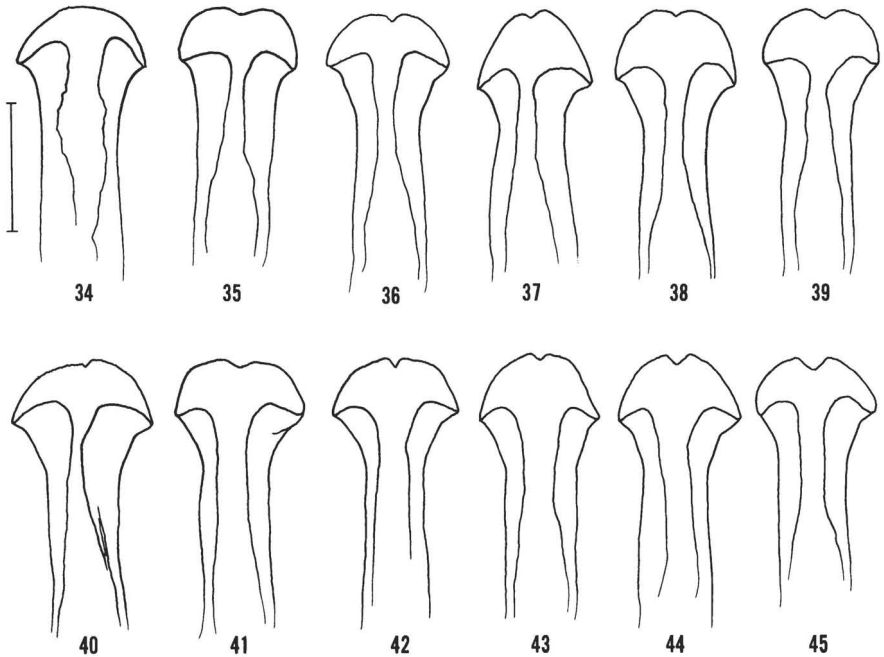
Johnson (1985:120–121) distinguished *M. millerorum* from *M. spinetorum* using shape of the genital plate and ventral ductus bursae. Differences “are most apparent in the nature of the lamellar [sic] lips caudad on the ductus bursae, the nature of the sclerotizations surrounding these lips, and the shape of the sculpturing caudo-ventrad on the ductus bursae.” However, shape of the posterior bursa copulatrix in specimens with and without a ventral hindwing basal line varied similarly (Figs. 34–45) and provided no evidence for two species in the Weed sample.

### Comparison with Types

The type of *M. spinetorum*, a female housed in the Smithsonian Institution, lacked the ventral hindwing basal line, and the angle formed by the postmedian line between veins Cu2 and 2A was  $107.51^\circ$  ( $n = 4$ ,  $s = 2.863$ ). Its genitalia (Fig. 42) and postmedian line angle fall within the range of variation of the Weed population.

The ventral wing pattern and genitalia of the female type of *M. millerorum* were clearly illustrated (by A. C. Allyn, J. Y. Miller) in Clench (1981). It possessed the ventral hindwing basal line, and its postmedian line angle was  $112.49^\circ$  ( $n = 4$ ,  $s = 8.810$ ). Its genitalia fall within the range of variation illustrated in this paper (compare Figs. 34–45 with fig. 36 in Clench 1981).

In accord with Scott’s (1986) hypothesis, *M. millerorum* Clench is a synonym of *M. spinetorum* (Hewitson). The wing pattern and genitalic characters proposed to distinguish these species (Clench 1981, Johnson 1985) vary continuously in the Weed population and are unimodal. The types of *M. spinetorum* and *M. millerorum* fall within the range of variation of this population. Thus, there is no published evidence



FIGS. 34-39. Posterior bursa copulatrix (ventral aspect) of specimens with a ventral hindwing basal line. Scale 1 mm.

FIGS. 40-45. Posterior bursa copulatrix (ventral aspect) of specimens without a ventral hindwing basal line from the Weed population, except for Fig. 42, which is the type of *M. spinetorum* from California.

supporting the hypothesis that *M. millerorum* is distinct from *M. spinetorum*.

#### DISCUSSION

##### Genitalic Differences in *Mitoura*

Johnson (1976, 1978, 1981, 1985) characterized North American *Mitoura* species using genitalic differences, but his results are invalid because he did not assess variation. Brown (1983) examined two male and two female genitalic preparations for each of four Californian *Mitoura* taxa and found greater variation within species than Johnson had reported between species. Johnson listed seven genitalic differences between *M. millerorum* and *M. spinetorum*, but these differences represent a small portion of continuous unimodal intraspecific variation.

TABLE 1. Observed and expected number of specimens of *Mitoura spinetorum* complex from Weed, New Mexico, with and without a ventral hindwing basal line when presence is determined by a sex-linked recessive in Hardy-Weinberg equilibrium (Crow & Kimura 1970). The maximum likelihood estimate for gene frequency is 0.170 and for proportion of males is 0.34.

	Males	Females
With ventral hindwing basal line		
Observed	2	13
Expected	1.3	14.3
No ventral hindwing basal line		
Observed	42	71
Expected	42.7	69.7

### Genetic Basis of Ventral Hindwing Basal Line

Two simple genetic mechanisms might account for the greater frequency of ventral hindwing basal line phenotypes in females (15.5%) than in males (4.5%). First, expression of the line may have a quantitative genetic basis with greater penetrance in females. Wing spots in *Erynnis* Schrank (Burns 1964) are an analogous example. Some spots in *Erynnis* may be present or absent, but are present more frequently in females. Further, spot size varies continuously. The variable expression of the ventral hindwing basal line in *M. spinetorum*, when present, is consistent with this mechanism.

A second possible mechanism is that expression of the line is determined by a sex-linked recessive allele in Hardy-Weinberg equilibrium. Following calculations in Crow and Kimura (1970:41-42) for a sex-linked recessive in which the female is the heterogametic sex, expected frequencies of phenotypes closely match observed frequencies (Table 1,  $\chi^2 = 0.57$ ,  $df = 1$ ).

These hypotheses on the genetic basis of hindwing basal line expression can be tested by data from rearing. Dimorphic populations are found at different localities in the Sacramento Mountains, and a New Mexican specimen with a hindwing basal line was collected by R. Holland on the crest of the Caballo Mountains (Sierra County).

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