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SEX-RELATED COLOR PATTERNS IN ELYTRAL VITTAE OF *DIABROTICA VIRGIFERA VIRGIFERA* (COLEOPTERA: CHRYSOMELIDAE)Louis S. Hesler<sup>1</sup> and Leslie Hammack<sup>2</sup>

## ABSTRACT

We evaluated the color patterns of elytral vittae by sex in adults of *Diabrotica virgifera virgifera* LeConte. Our study examined >1000 beetles taken from a field population, a laboratory colony, and a reference collection containing 712 specimens from 15 of the United States and from the province of Ontario, Canada. The humeral and sutural vittae of each beetle's elytra were classified as being separate, partially confluent, or totally confluent with each other. The distribution of these elytral patterns was not independent of sex. Males tended to have confluent or partially confluent vittae, whereas females largely had separate vittae. Nonetheless, all three patterns of elytral vittae were found in both sexes of *D. v. virgifera*, and many beetles of each sex had partially confluent vittae. This data shows that sexing *D. v. virgifera* beetles by simple examination of elytral vittae alone is unreliable.

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The western corn rootworm, *Diabrotica virgifera virgifera* LeConte, is a major pest of maize (*Zea mays* L.) in the United States. Maize producers lose over \$1 billion annually in control costs and yield reduction because of *D. v. virgifera* and other rootworm species such as *D. v. zea*, *D. barberi*, and *D. undecimpunctata* (Metcalf 1986).

Like other members of the genus, *D. v. virgifera* is sexually dimorphic externally (White 1977). Males are distinguished most reliably from females by the presence of an additional abdominal tergite (Krysan 1986). However, distinguishing sexes based on the number of abdominal tergites requires examining pinned or anesthetized specimens with 10x or greater magnification (Krysan 1986).

Antennal characters also differ between the sexes of *D. v. virgifera*. Relative antennal length is greater in males (nearly as long or longer than the body) than in females ( $\frac{1}{2}$  the body length) (Krysan & Smith 1987). Some experienced workers use relative antennal length to differentiate the sexes superficially (Krysan 1986), but others are uncomfortable with their proficiency in using this character.

Additionally, antennal flagellomeres 2 and 3 are equal in length in males, but in females flagellomere 3 is distinctly longer than 2 (Krysan & Smith 1987). Like counting the number of abdominal tergites, sex determination

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based on the length of flagellomeres requires examining pinned or anesthetized specimens under magnification (Krysan 1986).

Finally, elytral coloration differs purportedly between the sexes of *D. v. virgifera* (Gillette 1912, Tate & Bare 1946, McLeod 1992, Steffey 1993). Each elytron of *D. v. virgifera* has two piceous vittae, one along the humeral margin and the other along the sutural margin (Krysan & Smith 1987). Putatively, in females the vittae are distinct, whereas in males the elytra are mostly piceous due to confluence of the humeral and sutural vittae (Gillette 1912, Tate & Bare 1946, McLeod 1992, Steffey 1993). This supposed sexual dimorphism in elytral vittae might provide a simple and rapid way to sex *D. v. virgifera*.

Krysan & Smith (1987) discussed the patterns of elytral vittae in *D. v. virgifera* but did not mention color pattern as a sexually based character. They did, however, mention that elytral vittae vary considerably in the *virgifera* subspecies, with gradations present in the degree of confluence (Krysan & Smith 1987). Substantial numbers of beetles with an intermediate color pattern of elytral vittae would invalidate a dichotomous method for sexing *D. v. virgifera*, unless the intermediate morphs were shown to be of one sex.

However, the validity of sexing adults of *D. v. virgifera* based on a dichotomy in elytral vittae is untested, except for a recent study in Virginia by Kuhar and Youngman (1995) that did not report the frequency of intermediate morphs. In our study, we (1) determined the frequency and distribution of elytral color patterns between the sexes of *D. v. virgifera* in specimens from 15 of the United States and the province of Ontario, Canada; and (2) tested the hypothesis that patterns of elytral vittae are independent of sex.

#### MATERIALS AND METHODS

Adult *D. v. virgifera* were obtained from three sources. First, beetles were collected from plants in three commercial maize fields in Brookings County, SD on 17 and 25 August and 9 September 1994. Each field was sampled on each date. Beetles were gently knocked off of maize plants from the ears and leaves into bottles that had been fitted with funnels. The beetles were taken to the laboratory and held in a freezer until they were processed.

The second source of adult *D. v. virgifera* was a laboratory colony from the USDA Northern Grain Insects Research Laboratory (NGIRL), Brookings, SD. The colony was established with beetles collected from a maize field in Brookings Co., SD, in 1987. One hundred beetles (49 females and 51 males) were extracted from the twelfth or thirteenth generation of the colony over 10 days during the period of adult emergence.

The third source was a reference collection housed at NGIRL. Using this collection, we examined 712 specimens of *D. v. virgifera* from 15 of the United States (AZ, CO, IA, KS, MN, MT, ND, NE, NM, OH, OK, SD, TX, UT, WY) and from the province of Ontario, Canada (ON). From one to 141 specimens were examined per state or province. Beetles from each state were usually collected from only 1-2 collection sites. At each site, beetles were generally collected within the same year, and often within one week of each other.

The elytral vittae of specimens from these three sources were examined. The vittae of each specimen were scored qualitatively as having (1) no or almost no confluence (separate), (2) considerable, partial confluence (partial), or (3) total or nearly total confluence (confluent). Kuhar and Youngman (1995) drew six elytral patterns ranging from the most striped to the most solid pattern, and then combined the first three patterns into a "striped" category and the last three into a "solid" category. The first two (most striped),

middle two, and last two patterns shown in Kuhar and Youngman (1995) correspond to our separate, partial and confluent categories, respectively. After scoring the confluence of a particular specimen, its sex was determined by examining the abdominal tergites, antennae, or both characters under a stereomicroscope. Data from the reference collection were subjected only to descriptive statistics. Data from the field and colony populations were used to test the hypothesis that the distribution of elytral patterns is independent of sex (contingency chi-square test, Zar 1984).

## RESULTS AND DISCUSSION

Data pooled from the three field populations of *D. v. virgifera* did not support the hypothesis that elytral patterns are independent of sex (Table 1;  $\chi^2 = 127.19$ ,  $df = 2$ ,  $P < 0.005$ ). Rather, the distribution of males was skewed toward the confluent and partial elytral patterns (31 and 28 of 75 males, respectively), whereas the distribution of females was skewed strongly toward the separate elytral pattern (201 of 233 females).

Similarly, data from the NGIRL colony did not support the hypothesis that elytral patterns are independent of sex (Table 2;  $\chi^2 = 69.33$ ,  $df = 2$ ,  $P < 0.005$ ). Nearly two-thirds of the males in the colony (33 of 51) had the confluent pattern. The distribution of females was skewed opposite toward the separate pattern (40 of 49 females). No female sampled from the colony had a confluent elytral pattern.

Similar trends were generally seen in specimens of *D. v. virgifera* from the reference collection (Table 3). Overall, slightly more than half (213 of 382) of the males examined had a confluent pattern. The remainder of male specimens were distributed almost evenly between partial and separate pat-

Table 1. Distribution of patterns of elytral vittae in each sex of adult *D. v. virgifera* collected from commercial maize fields in Brookings County, SD, 1994.

Sex	No. with indicated pattern		
	Separate	Partial	Confluent
Males	16	28	31
Females	201	26	6

Elytral pattern was not independent of sex ( $\chi^2 = 127.19$ ,  $df = 2$ ,  $P < 0.005$ ). Data from three fields each sampled on different dates (17 and 25 August and 9 September) were pooled after establishing that field did not affect the proportions of the populations with the three elytral patterns ( $\chi^2 = 3.87$  and  $7.44$  for males and females, respectively,  $df = 4$ ,  $P > 0.1$ ).

Table 2. Distribution of patterns of elytral vittae in adult *D. v. virgifera* sampled from a laboratory colony.

Sex	No. with indicated pattern		
	Separate	Partial	Confluent
Males	2	16	33
Females	40	9	0

Elytral pattern was not independent of sex ( $\chi^2 = 69.33$ ,  $df = 2$ ,  $P < 0.005$ ).

Table 3. Patterns of elytral vittae of adult *D. v. virgifera* collected from various states and the province of Ontario.

State	Sex	Elytral pattern		
		Separate	Partial	Confluent
AZ	Males	0	0	1
	Females	21	3	3
CO	Males	3	0	3
	Females	5	1	0
IA	Males	1	0	0
	Females	4	1	0
KS	Males	5	5	48
	Females	30	3	6
MN	Males	0	2	5
	Females	3	0	0
MT	Males	6	0	0
	Females	4	0	0
ND	Males	3	1	0
	Females	8	0	0
NE	Males	3	3	17
	Females	43	3	8
NM	Males	1	2	3
	Females	5	0	0
OH	Males	2	0	2
	Females	3	0	3
OK	Males	0	0	0
	Females	1	0	0
SD	Males	4	4	20
	Females	50	1	5
TX	Males	33	27	22
	Females	32	1	3
UT	Males	9	23	47
	Females	34	13	15
WY	Males	0	1	0
	Females	1	0	0
ON	Males	14	17	45
	Females	16	0	1
TOTAL	Males	84	85	213
	Females	260	26	44

terns (85 and 84 specimens, respectively). The distribution of elytral patterns in females was strongly skewed toward the separate pattern (260 of 330 females). Twenty-six and 44 of the remaining 70 females had the partial and confluent patterns, respectively. Interestingly, a large proportion of males (33 of 82, or 40%) from TX had a separate elytral pattern. Also, nearly half of the 62 females from UT had either a confluent (15) or partial elytral (13) pattern.

Our results show that elytral patterns of *D. v. virgifera* are not simple, dichotomous, and sex specific. Rather, our data show that all three elytral patterns are found in both sexes of *D. v. virgifera* and that the distribution of these patterns is geographically widespread. However, our results do show that the distribution of patterns of elytral vittae is sex-related: males tend to have confluent or partially confluent vittae and females largely have separate vittae.

Our results contrast with previous publications that purport elytral color patterns in *D. v. virgifera* to be simple and sex specific (Gillette 1912, Tate & Bare 1946, McLeod 1992, Steffey 1993). Statements about elytral patterns in these publications may be oversimplifications of sex-related trends in the patterns of elytral vittae shown by our data.

The results of our study both agree and contrast with those of Kuhar and Youngman (1995), who classified the elytral patterns of *D. v. virgifera* adults as either striped or solid. Consistent with our results, they found striped and solid elytral patterns in both sexes of *D. v. virgifera* in Virginia. They also found that the sex of striped beetles could not be determined accurately by simple visual inspection. However, they concluded that solid morphs could reasonably be considered males, as males comprised >98% of all solid morphs. In contrast, we found that about 15% of confluent morphs were female in a geographically more diverse sample.

Our results are relevant to pest management practitioners and researchers, who often need to determine the sex of large numbers of *D. v. virgifera* adults. For instance, knowing the percentage of female *D. v. virgifera* adults in maize fields at critical times may be important in implementing control measures that prevent egg-laying by this pest (Sutter and Lance 1991). Also, the evaluation of semiochemicals and insecticides for *D. v. virgifera* sometimes requires sexing hundreds to thousands of adult beetles, because the sexes can respond differently to such chemicals (Sutter et al. 1990, Hesler et al. 1994, Hammack & Hesler 1995). Thus, there is a need for a simple and rapid method for sexing *D. v. virgifera* adults. However, because we have shown that elytral patterns are unreliable for accurately sexing *D. v. virgifera* adults, we recommend examining abdominal tergites as the best alternative (Krysan 1986).

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