PIGMENTATION ASSOCIATED WITH OOGENESIS IN THE BITING MIDGE, *CULICOIDES VARIIPENNIS*: CHANGES IN ABDOMINAL TERGITE PATTERNS

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ABSTRACT. Patterns of pigmentation in abdominal tergites 2 and 3 of adult female *Culicoides variipennis* (Coquillett) were examined for changes associated with blood feeding. Six tergite types were identified that allowed separation of nulliparous from parous flies based on pigmentation. In colonies of flies from Texas and Idaho, nulliparous flies had open posterior margins that contained 2 long unpigmented areas that extended anteriorly nearly the length of the tergite; field-collected flies from Colorado had an additional pigmented spot in each unpigmented area. Patterns that developed after a blood meal were characterized by closed posterior margins that appeared smooth or slightly wavy. These patterns were developed before the blood meal was fully digested and remained constant for the life of the fly; additional blood meals did not change the types. The classification of tergite types in *C. variipennis* was easy, worked with alcohol-preserved flies without sugar meals, and distinguished parous from nulliparous females with 98% accuracy.

INTRODUCTION

The biting gnat *Culicoides variipennis* (Coquillett), a widespread pest of ruminant animals in the United States, is the primary vector of bluetongue and epizootic hemorrhagic disease viruses (Jones et al. 1977). Studies of the epizootiology of these diseases require reliable methods of determining rates of parity for female *C. variipennis* because the ability of a population of *C. variipennis* to transmit diseases is dependent on the number of infected females available to seek blood meals. In addition, a rapid method of determining parity would enable investigators to quickly sort out parous flies for virus assay.

Several methods have been used to determine parity in *Culicoides*, e.g., ovarial dilatations, ovarial color differences (Dyce 1969), ovarial tracheal patterns (Akey and Potter 1979), and the increase in ventral abdominal pigmentation first described by Dyce (1969). The latter is the easiest of the 4 methods to use. However, while studying the relationship of abdominal pigmentation to parity rates in *C. variipennis* (Akey and Potter 1979), we also observed differences in the pigmentation patterns of the abdominal tergites. The purpose of the present study was to characterize these differences and to relate them to parity in *C. variipennis*.

METHODS AND MATERIALS

All adult female flies were held at 24 ± 1°C and 30–40% RH with a 16L:8D photoperiod and provided with 10% sugar solution or water.

Colony *C. variipennis* that originated in Texas (Sonora Strain, 000 line, Jones et al. 1969), was studied first to classify and describe the development of the abdominal tergite (AT) patterns. In this investigation, the females were blood fed when they were 24 to 48 hr old. Flies were then killed at 6-hr intervals during the first day after blood feeding and at 2, 3, 5 and 8 days. Control flies (no blood meal) were killed at 1, 7, 15 and 26 days after emergence to determine whether any changes in pattern occurred with age.

Next, flies from a colony that originated in Idaho (Bruneau strain, 036 line, Jones and Foster 1978) and flies that were field collected as pupae near Wattenberg, Colorado (Jones and Akey 1977) were compared with the colony flies from Texas to determine whether AT patterns for the 3 populations were similar; com-

parisons were made either 1 day after emergence (control flies) or at 5 days after blood feeding.

Flies were usually preserved in 70% ethyl alcohol, and AT patterns were later examined with a stereomicroscope at 12 or 20X magnification. Dead flies were occasionally removed from the cages and examined to determine whether the AT patterns could still be detected on their dry, shriveled abdomens.

RESULTS

Microscopic examination of the abdomens of female *C. variipennis* that had blood fed showed changes in AT 2, 3, and 4; no change was seen in the pattern of AT 1. We therefore used the patterns in AT 2 and 3 as the basis for the classification of types. Photographs of tergite types are presented in figures 1 and 2. Figures 3 and 4 are stylized line drawings and are useful as a guide to the following classifications:

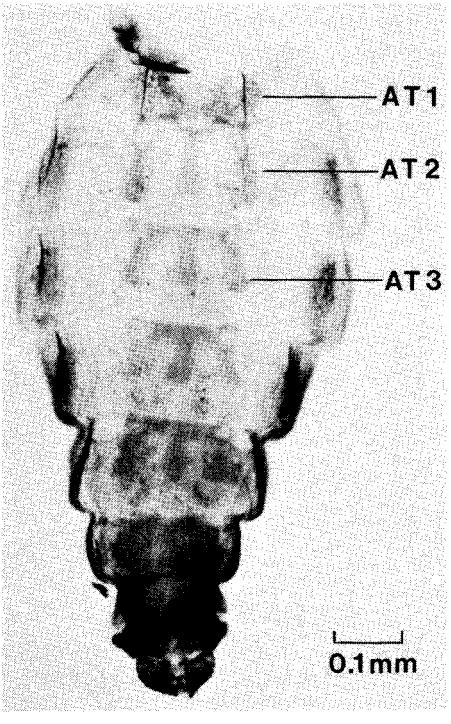

Figure 1. Whole mount of abdomen of nulliparous *Culicoides variipennis*, 2 days after emergence, with a deeply indented abdominal tergite (AT) on segments 2 and 3. Type I.

A. NULLIPAROUS CONDITION

Type I: Posterior margin with 2 long unpigmented areas in AT 2 and AT 3 (Fig. 1,3A). The pattern at AT 2 appeared as 3 long, narrow, pigmented areas connected anteriorly. The posterior margin of the pattern was open and contained 2 long, unpigmented areas that extended nearly the length of the tergite and were as large as the pigmented areas. The posterior margin of AT 3 was similar to that of AT 2, but the anterior connection was much wider so the length of the unpigmented areas in the posterior margin of the pattern was ca. ¾ the length of the tergite. Of the 200 field-collected flies from Wattenberg, Colorado, 157 (78%) had small pigmented spots, either attached or detached near the apical end of the median pigmented portion; these spots were indistinct in colony flies.

B. INTERMEDIATE CONDITION DURING OOGENESIS

Type II: Posterior margin with 2 moderately long unpigmented areas in AT 2 and AT 3 (Fig. 3B). The pigmented areas in AT 2 were broader than in Type I; the unpigmented areas were narrower. The posterior margin of AT 3 contained small unpigmented areas ca. ¼ the length of the tergite.

Type III: Posterior margin closed and circular unpigmented areas in AT 2; posterior margin with slightly moderate indented patterns in AT 3 (Fig. 2A,3C). The posterior margin of AT 2 was closed so that there were 2 circular unpigmented areas medially. The posterior margin of AT 3 was similar to that of AT 3 in Type II.

C. PAROUS CONDITION

Type IV: Posterior margin closed and circular unpigmented areas in AT 2; entirely

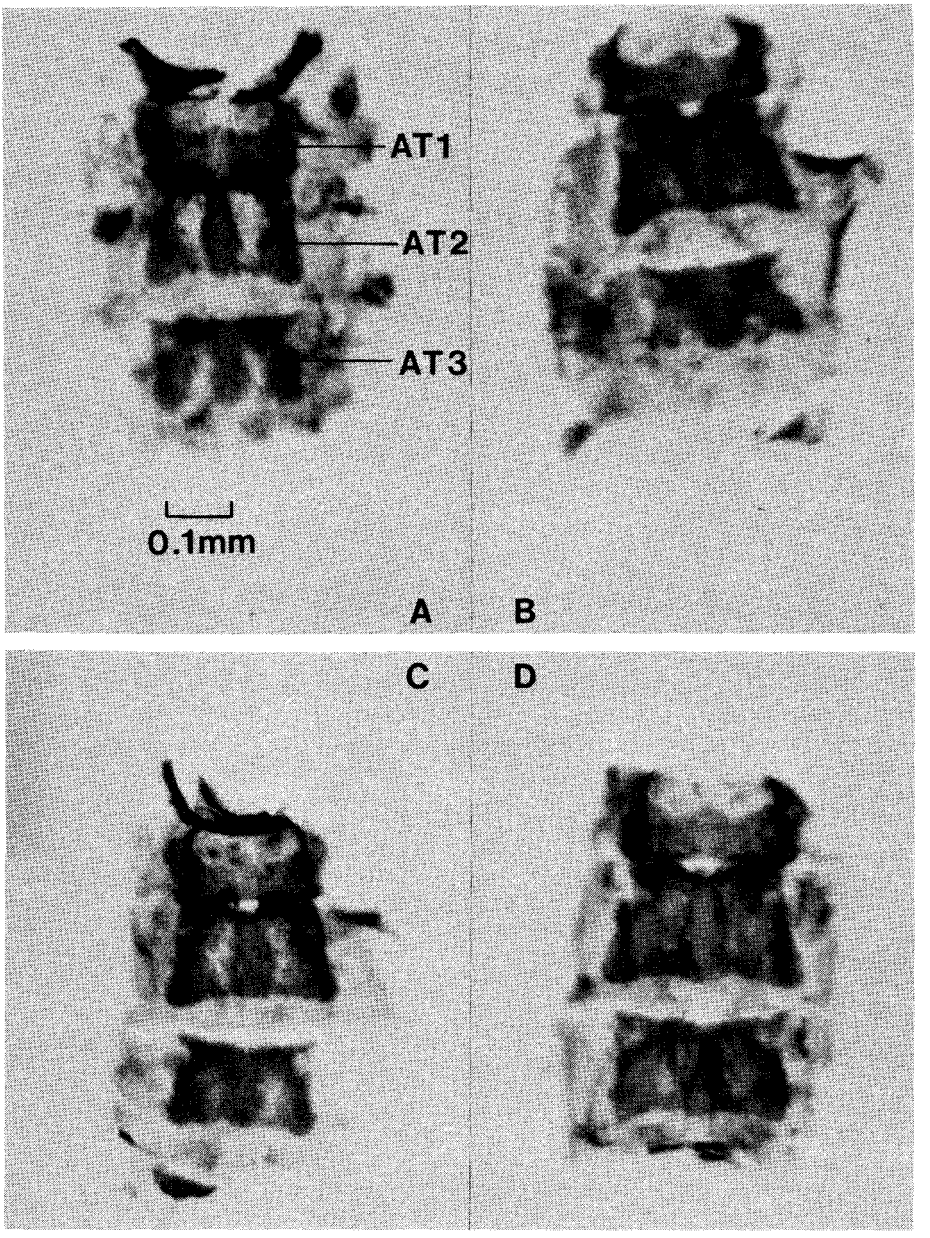

Figure 2. Abdominal tergites (AT) of segments 1, 2 and 3 excised from parous *Culicoides variipennis* females 5 days after blood feeding. A. Type III, B. Type IV, C. Type V, D. Type VI.

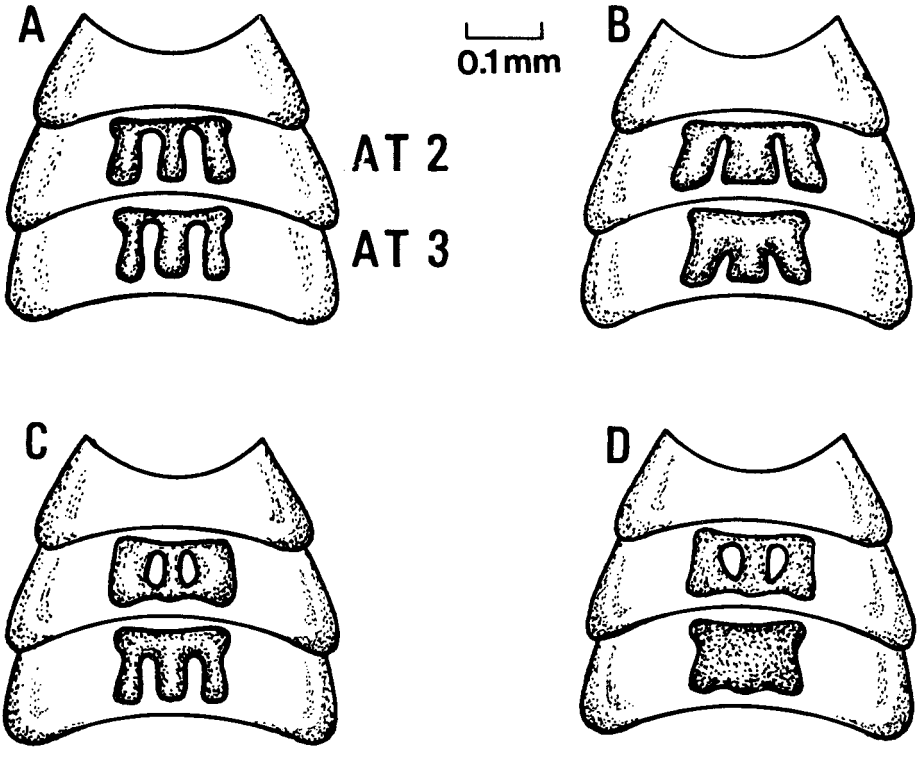

Figure 3. Stylized line drawings of abdominal tergites (AT) 2 and 3 of female *Culicoides variipennis*. A. Type I, B. Type II, C. Type III. D. Type IV.

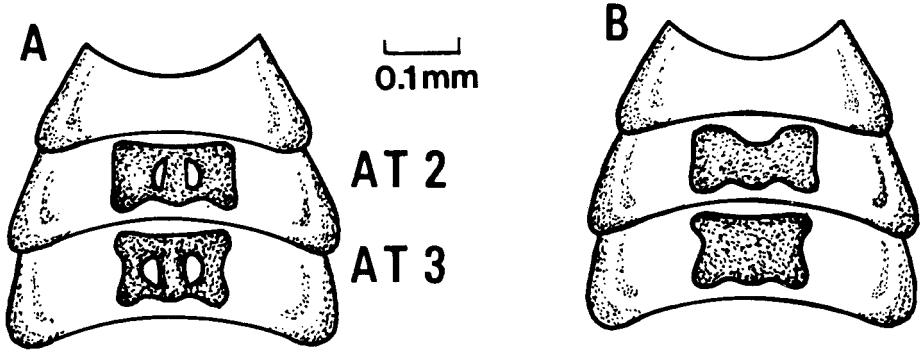

Figure 4. Stylized line drawings of abdominal tergites (AT) 2 and 3 of female *Culicoides variipennis*. A. Type V, B. Type VI.

pigmented AT 3 (Fig. 2B, 3D). Pigmented areas of AT 2 contained 2 unpigmented areas medially as in Type III. The center of AT 3 was entirely pigmented, and the posterior margin was either smooth or slightly wavy.

Type V: Posterior margin closed with circular unpigmented areas in AT 2 and AT 3 (Fig. 2C,4A). The posterior margin of both AT 2 and AT 3 was closed. Each tergite contained 2 circular unpigmented areas as in Type III AT 2. The posterior margins were either smooth or slightly wavy.

Type VI: Entirely pigmented AT 2 and AT 3 (Fig. 2D,4B). AT 2 and AT 3 had pigmentation throughout the entire tergite. The posterior margins of both tergites were either smooth or slightly wavy.

Once the types were defined, we used them to sort nulliparous from parous flies. We found that a combination of AT 2 and AT 3 gave the most accurate determinations for parity. When Type I was used to indicate nulliparity and all other types were used to indicate parity, the sorting error was 0.2% (1/491) for nulliparous flies and 1.5% (9/607) for parous flies (Table 1). When either AT 2 or AT 3 were examined separately, the existence of the closed posterior margin and 2 circular unpigmented areas medially in both nulliparous and parous flies made 22% of the determinations of parity uncertain (Table 1). The pattern in AT 4 was not used because it made the classification needlessly complex and did not increase accuracy.

Type I was found predominantly in

Table 1. Abdominal tergite types for different ages of female *C. variipennis* with and without blood meals.

Source	Age[b] (days)	Classification of abdominal tergite types[a] (Numbers of flies)						
		Type I	Type II	Type III	Type IV	Type V	Type VI	Total
				WITH A BLOOD MEAL				
Colony (Texas)	.25	10		3	15	6	4	38
	.50	1			5		35	41
	.75		3	3	11	1	22	40
	1				9	3	24	36
	2			8	5		40	53
	3			3	7	4	47	61
	5	1	5	25	19	31	113	194
	8			3	7	4	40	54
Colony (Idaho)	5		1	5	31	7	29	73
Wattenberg, CO	5			1	4	12		17
		12	9	51	113	68	354	607
				WITHOUT A BLOOD MEAL				
Colony (Texas)	1	59[c]				1		60
	8	24				1		25
	15	154				4		158
	25	10						10
Colony (Idaho)	1	33						33
Wattenberg, CO	1	202				3		205
		482				9		491

[a] Full description of Types I-VI is given in text.
[b] Age after blood meal or age after emergence for flies without a blood meal.
[c] Includes 30 female flies removed immediately after blood feeding.

nulliparous flies; it persisted throughout their lives. Types II and III, which appeared 6 hr after a blood meal, were intermediate stages in the development of tergite pigmentation. Only 1.9% of the flies had Type II pigmentation remaining 18 hr after blood feeding, and only 11% of the flies had Type III remaining at 3 days after blood feeding.

Types IV, V and VI were the final stages that developed after blood feeding. The changes were complete before the blood meal was fully digested, that is 3–4 days after feeding, and sometimes they were developed within 6 hr after blood feeding. However, 2% of the flies that had not taken a blood meal were Type V and could not be differentiated from blood-fed Type V flies. Once the final tergite type developed, it was not influenced by additional blood meals.

Classification of flies that died and air dried was sometimes uncertain because as the abdomens shriveled, the patterns in the tergites became obscure.

DISCUSSION

The 3 different populations of *C. variipennis* had similar AT patterns before they received a blood meal. However, field-collected flies frequently showed an additional pigmented area that was indistinct in colony flies. Thus a study of the AT patterns in flies before they receive a blood meal might be of use in establishing differences among populations, subspecies, or species. Also the final pattern reached in blood-fed flies might be useful as a genetic marker.

Parity in *C. variipennis* can be determined as easily by AT types as by abdominal pigmentation. Neither method requires the dissection time needed for ovarial examination and both methods allow for assay of flies that are undamaged by dissection. However, AT patterns can be observed in flies that do not have their abdomens distended by a sugar meal; a sugar meal aids observation of abdominal pigmentation. Also tergite patterns can often be seen in dead, shriveled flies when abdominal pigmentation cannot be observed. AT types are more accurate than abdominal pigmentation for the determination of parity (98% vs. 92%; Akey and Potter 1979).

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References Cited

Akey, D. H. and Potter, H. 1979. Pigmentation associated with oogenesis in the biting fly *Culicoides variipennis* (Diptera: Ceratopogonidae): Application as an age grouping method. J. Med. Entomol. (In press).

Dyce, A. L. 1969. The recognition of nulliparous and parous *Culicoides* (Diptera: Ceratopogonidae) without dissection. J. Aust. Entomol. Soc. 8:11–15.

Jones, R. H. and Akey, D. H. 1977. Biting flies attacking Holstein cattle in a bluetongue enzootic area in Colorado, 1976. Mosquito News. 37:372–375.

Jones, R. H. and Foster, N. M. 1978. Relevance of laboratory colonies of the vector in arbovirus research—*Culicoides variipennis* and bluetongue. Amer. J. Trop. Med. Hyg. 27:168–177.

Jones, R. H., Potter, H. W. and Baker, S. K. 1969. An improved larval medium for colonized *Culicoides variipennis*. J. Econ. Entomol. 62:1483–1486.

Jones, R. H., Roughton, R. D., Foster, N. M. and Bando, B. M. 1977. *Culicoides*, the vector of epizootic hemorrhagic disease in white-tailed deer in Kentucky in 1971. J. Wild. Dis. 12:2–8.