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Attachment and silk of larvae of *Simulium vittatum* Zetterstedt (Diptera: Simuliidae)

by

W.B. Barr

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF Master of Science

Department of Entomology

EDMONTON, ALBERTA
Fall, 1982

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Attachment and silk of larvae of *Simulium vittatum* Zetterstedt (Diptera: Simuliidae) submitted by W.B. Barr in partial fulfilment of the requirements for the degree of Master of Science.

D. A. Craig
....
Supervisor

....

....

Date 8th Oct 1982

To my parents

Abstract

The success of Simuliidae is due in part to the combination of silk and hooks that their larvae employ to attach themselves in rivers and streams.

When attaching its anal proleg to a pad of silk, a *Simulium vittatum* larva first grasps the posterior end of its pad of silk with its thoracic proleg, then it grasps the anterior end of the pad with its mouthparts, and finally it grasps the middle of its pad with its anal proleg. Larvae then detach their mouthparts and thoracic proleg from the pad of silk to remain attached by the anal proleg alone. During this process, larvae bend their bodies laterally rather than dorso-ventrally; this probably allows larvae to keep their bodies in the slower current nearest the substrate.

Larvae produce wide filaments of silk probably by moving the salivary meatus anterodorsally during spinning, and by drawing silk out of the salivary meatus with their maxillae. Larvae probably grasp anchored lines of silk with their mandibular teeth. These teeth also swing past mental teeth closely, probably shearing off filaments of silk. Flanges of mental teeth probably also help to cut silk.

A pair of campaniform sensilla located posteriorly on the thoracic proleg, just outside the circlet of hooks, perhaps help larvae sense when the thoracic proleg is

attached securely. Bristles of lateral plates of the thoracic proleg probably help larvae to disengage hooks of the proleg from silk.

Tubercles on hooks of anal prolegs of larvae probably prevent those hooks from working loose from pads of silk and thus probably allow larvae to remain attached with minimal effort. The anal sclerite probably helps to attach hooks on the posterior half of the anal proleg to pads of silk.

An abandoned pad of silk has a c-shaped area where the thoracic proleg attached, a peaked area where the mouthparts attached, and a D-shaped area where the anal proleg attached. Pads of silk that larvae attach to while filter-feeding (feeding pads) have more definite structure and orientation, and contain more silk, than do pads of silk that larvae attach to while moving about (locomotory pads). Larvae do not eat silk they have detached from, but they conserve energy by producing relatively thin feeding pads and even thinner locomotory pads. Because pads rot, the rate of breakdown determines the maximum amount of time a larva can remain attached to a pad of silk before having to spin and attach to a new pad.

Differences in amino acid content between silk for larval attachment and silk for cocoons suggest that these two silks have different compositions suited to their different physical roles.

Inferences based on Hennig's (1973) reconstructed phylogeny of Culicomorpha suggest that the method of attachment of black-fly larvae arose through adaptation to feeding on substrates over which water flowed, and that the method was refined during a shift to passive filter feeding.

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I am grateful to D.A. Craig for his support (from NSERC Canada grants) and for his advice.

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G. Hutchinson analyzed water samples. Environment Canada Water Survey, Inland Directorate, supplied data on the Sturgeon River.

J. Nattriss performed amino acid analyses on samples of silk that C. Sanders had prepared. Both worked in L.B. Smillie's laboratory. The Department of Clothing and Textiles allowed me to use their polarizing microscope.

R.V. Peterson helped me learn to recognize *Simulium vittatum* larvae and pupae, and confirmed identification of *Simulium vittatum* larvae. D.C. Currie and A.P. Nimmo identified aquatic organisms.

General discussions with D.A. Craig, J.J.H. Ciborowski, and D.C. Currie stimulated and focussed my

thinking.

D.C. Currie suggested statistical analysis of development of silk. J.J.H. Ciborowski recommended the method of presenting data on proportions of rotting pads.

D.A. Craig suggested the function of tubercles on hooks of anal prolegs. The proposed mechanism for anal sclerite function emerged during discussion with G.A.P. Gibson. R.W. Smith helped to clarify function of posterior hooks of the anal proleg. J.N. Campbell suggested that silk could have an enzyme-resistant conformation. J.S. Ashe and G.E. Ball recommended ways of analyzing the evolution of attachment.

D.A. Craig, J.J.H. Ciborowski, B.K. Mitchell, R.B. Aiken, and J.S. Ashe criticized earlier drafts of various chapters. J. Scott gave advice on production of figures and on photography. H.E.L. Maw advised me about arranging text by computer. J.E. O'Hara and A.P. Nimmo suggested methods of drawing.

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List of Abbreviations

Abbreviation	Meaning
ab	apical brush of mandible
ac	circlet of hooks of anal proleg
acr	acrylic tubing
af	anal flap
ag	anterior gap in circlet of hooks
an	antenna
ao	lobe of anal organ
apb	anterior palatal brush
ar	tip of anterior arm of anal sclerite
as	sensilla of anal sclerite
at	apical tooth of mandible
ba	base of hook of proleg
bb	aboral maxillary brush
be	bend in labial gland
bi	bristle of lateral plate of thoracic proleg
br	brown silk
CA	calcium
ca	c-shaped area of pad of silk
cb	covering brush of mandible
cf	cephalic fan
cl	clear silk
cs	campaniform sensillum
ct	corner tooth of mentum
Da	D-shaped area of pad of silk
db	debris
di	diatom
dp	dorsal premental brush
ds	apicodorsal maxillary sensillum
e	closed end of labial gland
f	possessing lateral palatal brushes with many rows of short bristles
f'	possessing lateral palatal brushes with one row of long bristles
f''	lateral palatal brushes reduced or lost
fc	flexible cuticle
fl	flange of mental tooth
ft	flexible plastic tubing
g	spiracular gas exchange
g'	cuticular gas exchange
ga	gauze
gl	epithelium of labial gland
gr	groove in apical mandibular tooth
ho	hook of proleg
hp	anterolateral projection of hypopharynx

ht	tubercles on hooks of posterior proleg
hx	hypopharynx
i	muscular insertion
it1 through it3	first through third intermediate mental teeth
K	potassium
la	lacinia
lc	labropalatal comb
lh	larger hole in pad of silk
lp	labial palpus
lt	lateral mental tooth
lx	laterodorsal expansion of anal proleg
L1 through L3	furrows in abdominal cuticle
m	mentum
mb	median labropalatal brush
Md	dorsal muscle of thoracic proleg
MG	magnesium
ml	midline of anal sclerite
mp	mid-labropalatal brush
ms	microscope slide
mt	median tooth of mentum
Mv2	ventral muscle of thoracic proleg
M1, M2	muscles of thoracic proleg and anal sclerite
NA	sodium
nc	nerve cord
o	opaque silk
ot1 through ot4	first through fourth outer teeth of mandible
P	phosphorus
p	possessing anal prolegs
p'	possessing prothoracic and anal prolegs
p''	possessing fused prolegs with accessory sclerites
pa	peaked area of pad of silk
pat	preapical tooth of mandible
pb	lateral premental brush
pc	pigmented cuticle
pg	posterior gap in circlet of hooks
pl	lateral plate of thoracic proleg
pp	posterior labropalatal brush
pr	tip of posterior arm of anal sclerite
pu	pump
px	plate of acrylic
rg	ridge leading to peaked area of pad of silk

ri	rim of pad of silk
ro	area of pad of silk rotted away
r1 through r3	first through third rows of hypopharyngeal brush
S	sulphur
s	labial glands secrete mucuous
s'	labial glands secrete attachment silk
s''	labial glands secrete attachment silk and cocoon silk
sc	scales of anal sclerite
sh	smaller holes in pad of silk
si	silk
si a	narrow filament of silk produced when salivary meatus moved posteroventrally
si b	wide filament of silk produced when salivary meatus moved anterodorsally
sr	lateral serration of mentum
st	spinose teeth of mandible
S1 through S5	sensilla 1 through 5 of labial palpus
t	tear in anal flap
tc	thoracic circlet of hooks
th	thoracic proleg
tl	lacinial teeth
v	ventral premental brush
va	ventral adoral maxillary brush
vb	ventral aboral maxillary brush
vm	ventromentum
vp	ventral papilla
vt	variable transformer
x	salivary meatus

1. Introduction

Black-flies (Simuliidae) are important because the adults plague warm-blooded vertebrates (including humans) (Crosskey, 1973), and because the larvae contribute to the flow of organic energy in rivers and streams (McCullough *et al.*, 1979a and b). Part of the success of this family is due to the combination of silk and hooks that larvae employ to attach themselves to substrates in rivers and streams (Nachtigall, 1974a). This strong attachment allows larvae to passively filter particles of food from the flowing water (Chance, 1977). I present here results of my studies exploring the nature of this attachment.

Chapter 2 introduces the whole larva and the overall sequence of behaviour that results in attachment. Records on photographs and on videotape of larvae in a specially-designed apparatus show the behaviour in some detail.

Results of this first study confirm that mouthparts spin silk, attach to the silk temporarily, and cut the silk off. I infer how mouthparts do this in Chapter 3. Relative positions of mouthparts when protracted and retracted, and the shape of teeth and brushes on mouthparts are the bases for these inferences. The finding of silk on some mouthparts lends circumstantial support.

I infer how the thoracic proleg and the anal proleg attach to silk and detach from silk in Chapters 4 and 5. Relative positions of structures when protracted and retracted, and the shape of tubercles and spines provide bases for these inferences. The finding of silk on structures adds circumstantial evidence. The shape and movements of the prolegs observed in Chapter 2 help to guide some of the inferences.

Larvae leave pads of silk behind when they attach to new pads of silk. I interpret, in Chapter 6, features of pads of silk by comparing them with features of behaviour observed in Chapter 2 and with the sizes of mouthparts and prolegs found in Chapters 3, 4, and 5. I distinguish in the laboratory, pads of silk larvae attach to while filter-feeding from pads larvae attach to while moving about. Similarity of pads of silk spun in the laboratory to those sampled from the Sturgeon River allow me to tell the two kinds apart. The finding that pads of silk rot in the river and the measurement of the thickness of a pad permit me to estimate how much silk larvae require to replace rotting pads. I infer the rate of rot from the appearance of various stages of rot on pads on microscope slides kept in the river for various lengths of time.

Contents of labial glands of alcohol-preserved larvae can be transparently brown, or clear, or opaquely white (Chapter 6). A X^2 test of colour against larval maturity confirms that as larvae mature opaque silk replaces clear silk that earlier replaced brown silk. That no clear silk appears to reach the mouthparts, and lack of difference in amino acid content between a sample containing mostly clear silk and another containing mostly opaque silk implies that clear silk is a precursor of opaque silk. This all suggests that larvae attach to the substrate with brown silk and spin cocoons out of opaque silk. Difference of amino acid content between brown silk and opaque silk, together with differences in secondary physical properties, imply that each silk has special properties suiting it for its special function.

Chapter 2, on larval behaviour during attachment, contains a discussion of the origin of one feature of simuliid attachment, lateral bending of the body. Chapter 8 explores more fully the origins of this attachment. Hennig's (1973) reconstructed phylogeny of the families most closely related to Simuliidae, the Culicomorpha, provide a skeleton, and correlations of changes of structure and function with changes of habitat provide the bases for my inferences.

2. Looping

2.1 Introduction

A black-fly larva possesses an anterior (thoracic) and a posterior (anal) hook-wielding proleg. Larvae employ three methods for moving about. They can lower themselves downstream for up to a meter, on the end of a line of their silk anchored to the substrate (Tarshis and Neil, 1970). To move greater distances downstream, larvae can detach completely from the substrate and drift suspended in the water column (Rubtzov, 1964).

To move themselves a body's length or less away, larvae follow a process called "looping," wherein they apply silk to the substrate with their mouthparts, grasp it with their thoracic proleg or mouthparts or both, and, bending the body to bring the abdomen anteriorly, attach the anal proleg to the silk. After releasing mouthparts or thoracic proleg or both from the pad, larvae can loop again or filter-feed passively from this attachment. Only by looping can larvae move themselves upstream or across the current.

Previous accounts of looping, while agreeing in general, disagree in detail. According to Jobbins-Pomeroy (1916) and Planchon (1844), when larvae bend to bring the abdomen anteriorly, the anal proleg attaches beside the thoracic proleg. But according to Grenier (1949), the anal proleg attaches beside the thoracic proleg and mouthparts.

Puri (1925, p. 297) stated that the anal proleg attaches "...in front of..." the thoracic proleg. Smart (1934, p. 227) would state only that the anal proleg attaches "...close to the head..." Rubtzov (1962) illustrated the anal proleg attaching posterior to the thoracic proleg. Finally, Chance (1970) implied that the thoracic proleg was not necessarily attached at all during looping.

Grenier (1949), Smart (1934), and Tonnoir (1923) found that the mouthparts grasped silk spread on the substrate as larvae brought the anal proleg to this new pad of silk. Hinton (1955), Rubtzov (1962) and Puri (1925), on the other hand, suggested that the mouthparts did not attach in any way but only spun silk. Oddly, van Oye (1936) implied that the mouthparts bring the anal proleg to the pad.

Puri (1925) stated that the thoracic proleg detached at the same time as the anal proleg attached, while Smart (1934) found that larvae released their mouthparts and thoracic proleg only after the proleg had attached firmly.

Rubtzov (1962) showed a larva bending dorso-ventrally while looping, but it is difficult to tell from most other accounts whether larvae bent their bodies dorso-ventrally or laterally during looping. Rubtzov's (1962) diagram disagrees with Smart's description (1934, p. 227) of a larva "...lying along the substratum..." as it spun its pad of silk. If bending is lateral, as Smart (1934) hinted, larvae could be reducing drag on themselves by keeping their bodies in the slow current close to the substrate. Chance (1977) found that this "boundary layer" of slow water extended less than 2 mm above the substrate, and that larvae in an "avoidance reaction" had all of the body in this layer.

This chapter aims to resolve previous disagreements in accounts of looping of black-fly larvae.

2.2 Materials and methods

To observe and record behaviour of larvae in moving water, I built a special stage and an apparatus for moving water through the stage (Figure 1).

For the stage, two plates of acrylic held four microscope slides arranged as in Figure 1a. Each plate had at its centre a square hole. Grooves in the acrylic plates held the slides in place, and bolts connecting the plates held the stage together. Slides were glued to each other and to the acrylic plates with silicon sealant. A collar of acrylic tubing (inside diameter 25 mm) was glued to each plate.

One end of a 2 m length of clear, flexible Tygon R-3603 plastic tubing (inside diameter 19 mm; outside diameter 25 mm) was covered with plastic mesh (pore size 700 x 800 um; thread size 200 um and 160 um) and glued with silicon sealant inside the acrylic collar on the upstream side of the stage. The stage was held vertically by this collar, upstream side down, in a clamp held in turn in a Palmer 6 cm brass rack. This rack was fitted at right angles to two identical racks to allow three-dimensional movement of the whole stage. The third rack was clamped to a laboratory stand.

Further assembly of apparatus awaited addition of water and larvae. Two tanks (Figure 1) were filled with water from the sampling site (see Chapter 6). Compressed air was bubbled from a tube fed to the bottom of the upstream tank. *Simulium vittatum* larvae were collected at the sampling site and reared for up to ten weeks as described in Chapter 6. The stage and the upstream tube connecting the upstream tank to the stage were filled with water and larvae were dropped into the stage.

To complete the apparatus, one end of a second 2 m length of the Tygon tubing was covered with nylon gauze (pore size 140 um; filament size 80 um) and pushed inside the acrylic collar at the downstream end of the stage. A covering of parafilm sealed the joint. The stage and downstream tube were laid gently on the bench to fill the downstream tube, and the end was then stoppered. Finally, the stoppered end was unstoppered underwater in the downstream tank.

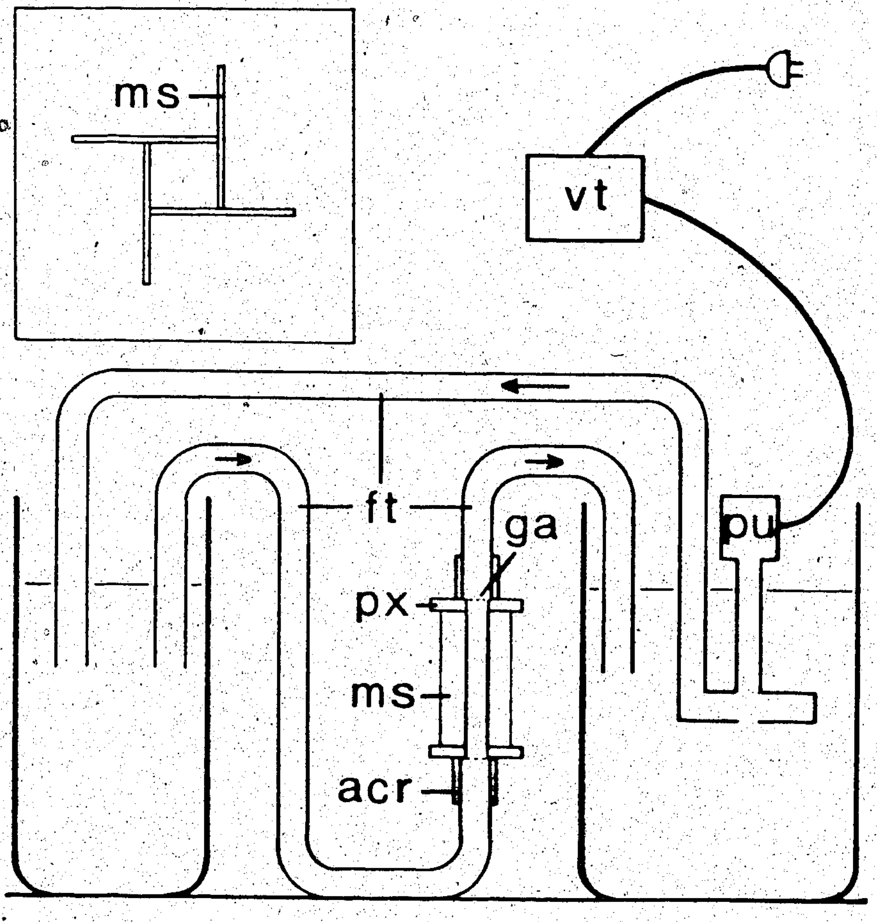
A centrifugal pump moved water from the downstream tank to the upstream tank. A variable transformer controlled the pump to keep the level in the upstream tank above that in the downstream tank. The difference in water levels drove water through the stage. Larvae filter-fed when differences in water level (head) exceeded 20 mm. Water velocity inside

Figure 1. Apparatus for recording behaviour of black-fly larvae.

Inset- detail of stage, cross section.

Arrows point in the direction of flow.

acr- acrylic tubing,
ft- flexible plastic tubing,
ga- gauze,
ms- microscope slide,
pu- pump,
px- plate of acrylic,
vt- variable transformer.



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the stage was not determined. Behaviour was recorded at water velocities created by water heads of up to 50 mm. Some larvae pupated inside the stage, and other larvae survived a 1 1/2-day test of the apparatus. To coax larvae to loop, the water head was changed.

To record behaviour inside the stage, a camera and a source of light were focussed at the same point, and the stage was moved to bring larvae into focus at that point. At times, intense light from the illuminators discouraged larvae from spinning silk. Larvae retracted lobes of their anal organs when the apparatus was rapped or scraped.

A Sony AVC 3200 black and white video camera was fitted by adaptors and Nikon extension tubes to a Zeiss Luminar 40 mm lens. The subject field at highest magnification was 3 mm wide, but most recording was done with the subject field 5 mm wide. A Javelin video recorder with Memorex 1/2-inch video tape recorded loopings in still water. A Mikrark 100W illuminator provided light.

An Ampex BCC4 color video camera was fitted with the same lens and extension tubes as the black and white camera. The subject field at highest magnification was 2.2 mm wide, but was 5 mm wide for most recording. A Sony videocassette recorder with Sony KCS20 3/4-inch tape recorded looping in still water. A Volpi Intralux 500H fiber optics illuminator provided light. Video tapes were played back on Sony 1/2-inch and 3/4-inch players and loopings were timed with a Heathkit electronic stopwatch.

Although nine sequences of larvae looping in the stage in still water were recorded on video tape, only one sequence of a larva looping in the stage in flowing water was successfully taped.

A Zeiss Luminar 40 mm lens on a Nikon PB-4 bellows extended 120 mm produced images magnified about three times on film in a Nikon FM camera. Loopings in the stage in still and flowing water were photographed at 1/60 sec and 1/120 sec on Kodak Ectachrome 160, Kodachrome 64 and Plus-X Pan 135 film. A Wild Heerbrugg high-intensity illuminator fitted with a concentrating lens provided light.

Drawings were made from projections of photographs of larvae onto paper. Additional observations by unaided eye were made of larvae in beakers of water stirred with magnetic stirrers.

2.3 Observations

Larvae began looping by closing their cephalic fans, bending their bodies laterally (Figures 2 and 4a), and attaching the thoracic proleg to the substrate (Figure 2). The thoracic proleg was bent at the joint between its two segments; the joint itself rested on the substrate. Thus only the ventral (posterior) part of the tip of the thoracic proleg grasped silk on the substrate. I could not tell whether the thoracic proleg pulled silk from the mouthparts and applied it to the substrate or if mouthparts applied silk alone and the thoracic proleg attached later. During one looping I observed, a larva detached its thoracic proleg as it brought its anal proleg to a new pad. This occurred in still water.

Anterior to the attachment of the thoracic proleg, larvae spun more silk on the substrate. As the mouthparts spun silk anterior to the thoracic proleg, the head moved anteriorly and posteriorly about one head-length. The head barely moved laterally, and did not roll. The mandibles or maxillae or both moved rapidly during spinning, but stopped moving when mouthparts grasped the pad at its anterior end. I could not tell which mouthparts grasped the pad to attach the head there.

Larvae then detached the anal proleg from the old pad and, bending the body laterally in a loop, attached to the new pad by inserting the anal proleg in the space between the head and thoracic proleg (Figure 3). As larvae brought the anal proleg to the new pad, the abdomen pushed the thorax to the side. Although this made the head pivot, neither the head nor the thoracic proleg released from the pad until after the anal proleg had attached. The head jerked dorsally when finally released from the pad.

In still water, larvae looped in an average of 2.2 sec (standard deviation 1.1; range 0.9 to 3.7 sec; nine observations on video tape), from when the thoracic proleg attached to the substrate until it released after the anal proleg had attached. The looping recorded in flowing water

Figure 2. *Simulium vittatum* larva spinning silk upstream of attachment of thoracic proleg, lateral view. Drawing from photograph.

Arrow points downstream.

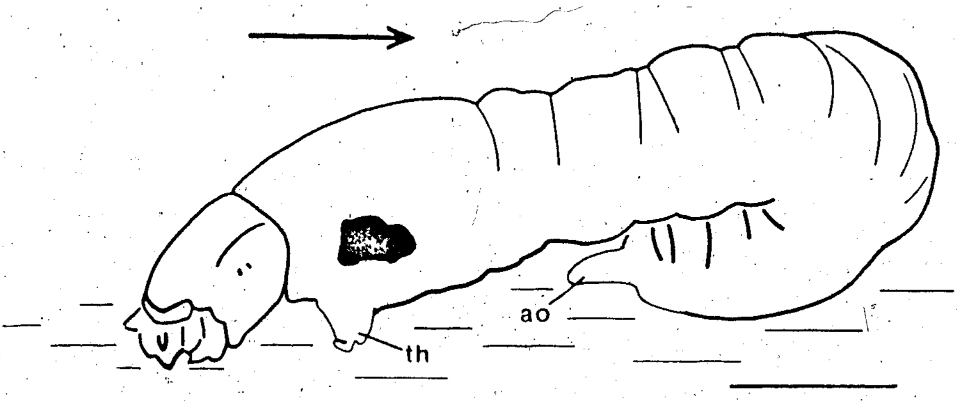
Scale=1 mm.

Figure 3. *Simulium vittatum* larva looping, ventral view. Having attached its anal proleg, larva has detached its mouthparts. Drawing from photograph.

Arrow points downstream.

Scale=1 mm.

ac- circlet of hooks of anal proleg,
an- antenna,
ao- lobe of anal organ,
fc- flexible cuticle,
nc- nerve cord.
th- thoracic proleg,



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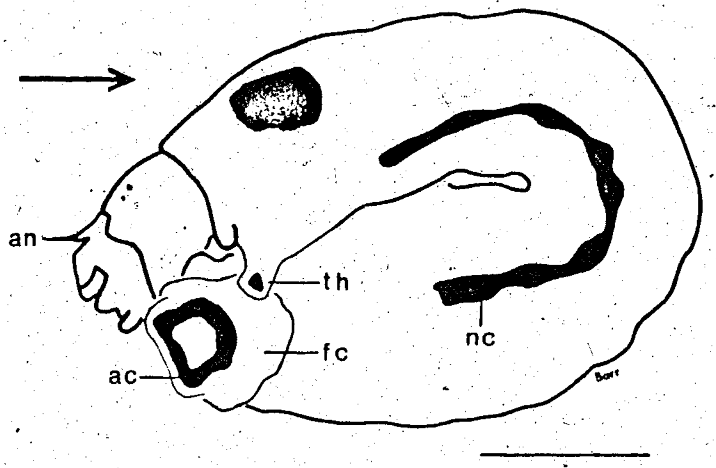

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took 2.1 sec from attachment to release of the thoracic proleg.

Often larvae attached the thoracic proleg twice to the substrate before attaching the anal proleg. Firstly, larvae grasped the substrate with the thoracic proleg as described, spun silk for a moment on the substrate ahead, grasped that silk with mouthparts and then regrasped with the thoracic proleg just behind where the mouthparts attached. This extended the body, and after regrasping, larvae spun a new pad of silk and looped as previously described. Sometimes larvae regrasped twice before attaching the anal proleg.

Perhaps noise of moving the stage about and bright light of illuminators distracted larvae from behaving exactly as they would naturally, but larvae undisturbed in the stage and larvae in beakers of water stirred with magnetic stirrers appeared, by naked eye, to behave no differently when looping. Mouthparts of larvae seemed to work longer at applying silk to the substrate when looping in flowing water than when looping in still water, but otherwise, whether looping in still or in flowing water, larvae appeared to loop by the same method. The following describes looping in more detail.

Larvae closed their cephalic fans before looping and opened them again only after finishing looping.

When attached, the anal circlet of hooks (ac, Figure 3) was D-shaped. The flat part of the D was where the posterior arms of the anal sclerite spread 180° apart, and

Figure 4. Diagram of larva looping upstream, dorsal view.

a. Larva spinning silk onto substrate.

Broken arrow points where abdomen moves when larva brings anal proleg from old pad of silk to new one.

b. Larva attaching anal proleg.

c. Larva filter-feeding in usual orientation.

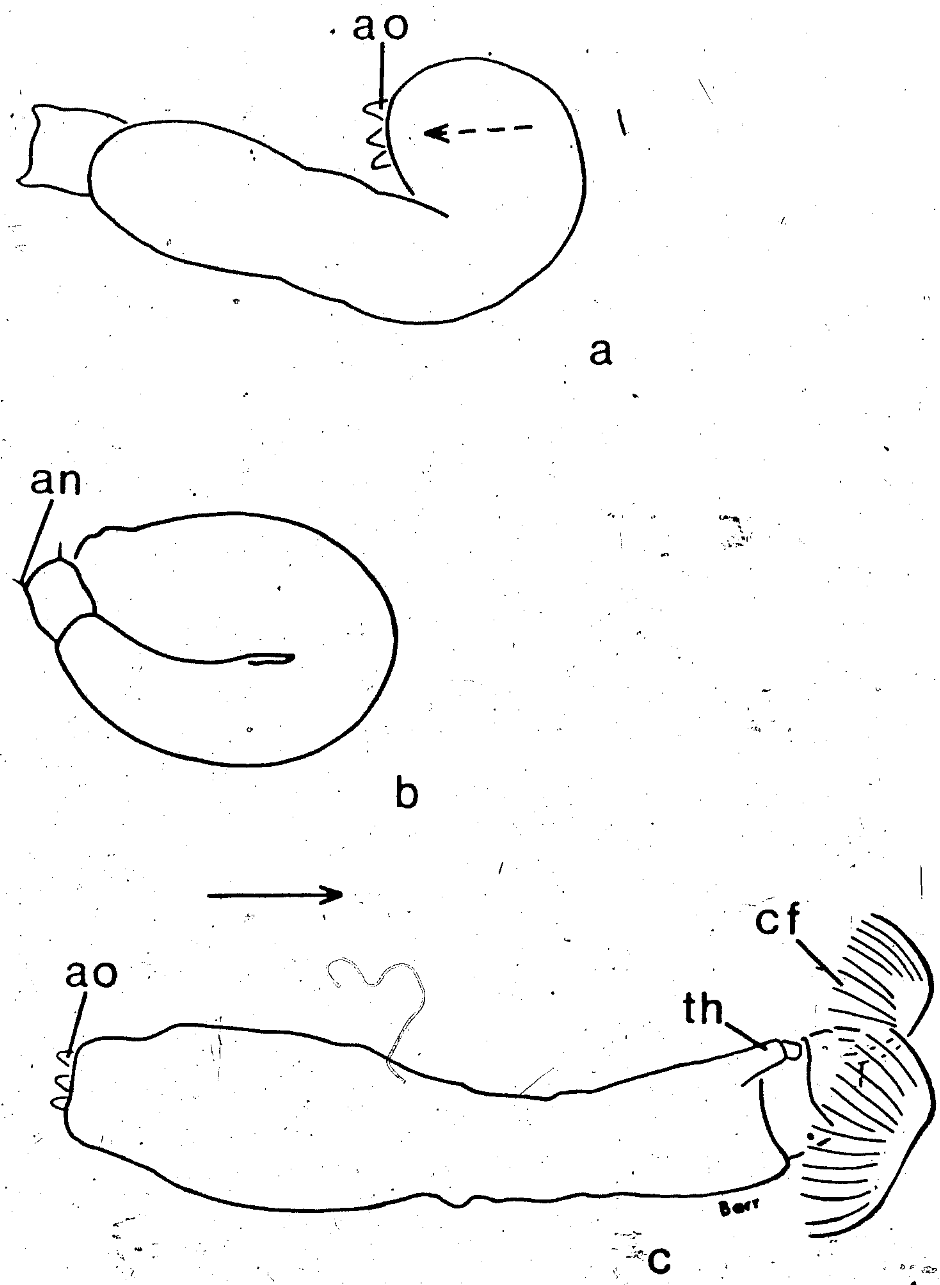
Solid arrow points downstream.

an- antenna,

ao- lobe of anal organ,

cf- cephalic fan,

th- thoracic proleg.



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was more or less upstream of the curved part of the D. The anal proleg and the cuticle around it was flexible (fc, Figure 3), allowing the proleg to twist more than 45°.

Before detaching their anal prolegs, larvae retracted their anal organs (ao, Figure 2), and did not protract them again until after releasing the head and thoracic proleg from the newly-spun pad. "Anal papillae" is the usual term for what I call the "anal organ" (Appendix Table A-9). In a high-magnification recording, the centre of the anal proleg could be seen retracting immediately before the anal proleg detached.

Turbulent currents buffetted filter-feeding larvae. Larvae absorbed shocks through keeping the head partly retracted in the thorax (Figure 4c). The pad of silk also appeared to absorb shocks, the whole anal circlet twisting quickly a few degrees, then bouncing back.

Filter-feeding larvae trail their bodies downstream of the attachment of the anal proleg (Figure 4c). In this orientation, lobes of the anal organ (ao, Figure 4c) point upstream.

Larvae did not always loop following the orientation described to this point. Larvae looping directly downstream (Figure 5) ended with their bodies opposite to usual orientation [note that the lobes of the anal organ (ao) in Figure 5c point downstream instead of upstream as in Figure 4c]. These larvae quickly looped again bringing themselves back to usual orientation: that is, body trailing downstream

Figure 5. Diagram of larva looping directly downstream, dorsal view.

a. Larva spinning silk onto substrate.

b. Larva attaching anal proleg.

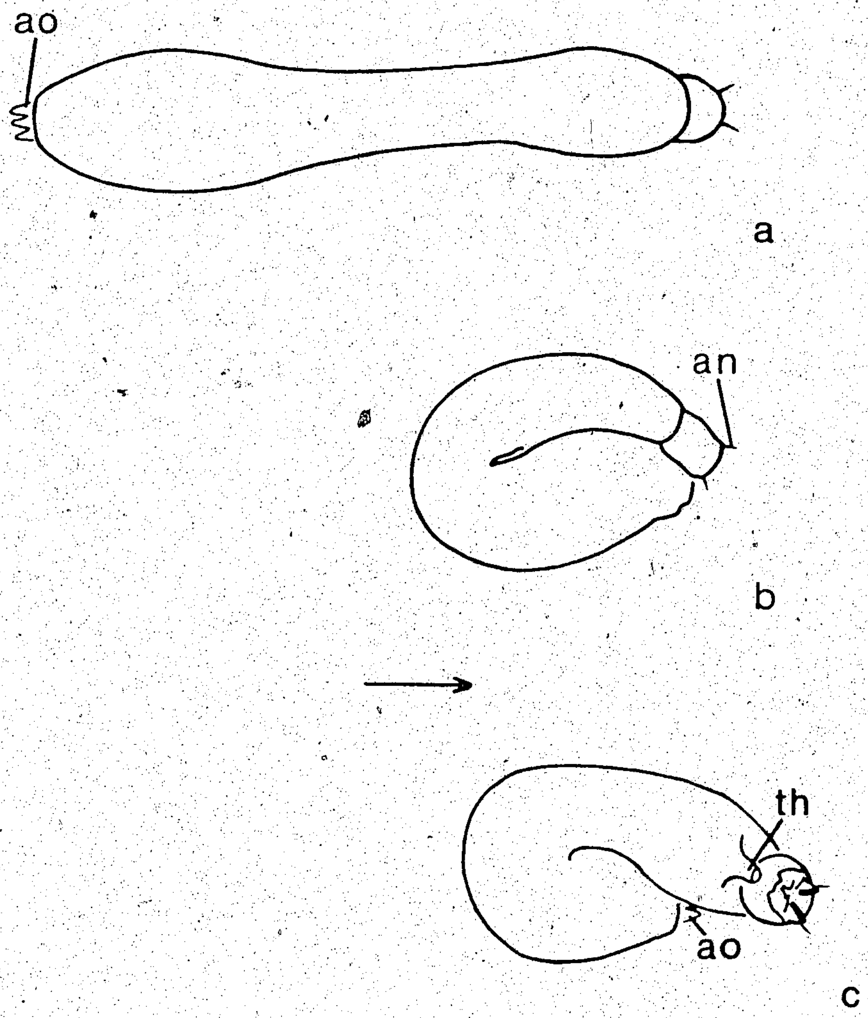
c. Larva attached, but opposite to usual orientation to current (Figure 4c).

Arrow points downstream.

an- antenna,

ao- lobe of anal organ,

th- thoracic proleg.



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of attached anal proleg, and papillae pointing upstream (Figure 4c).

Larvae looping laterally brought the anal proleg to the new pad in either of two ways (Figure 6). If they brought the anal proleg up directly, it attached in a poor orientation to the current (Figure 6b). Some larvae filter-fed from such attachments, with the supple anal proleg twisting to allow the larva's body to trail downstream. After detaching the anal proleg, larvae could also swing the abdomen downstream (Figure 6c) and around to the opposite side of the thorax (Figure 6d). After attaching, the anal proleg was well-oriented to the current, as a comparison of Figures 6d and 4b shows.

2.4 Discussion

More photomacrographic records of looping in flowing water should be made and studied to check my observations.

The present account of looping differs in at least one detail from previous accounts. Puri (1925) stated that the anal proleg attached "...in front of..." the thoracic proleg, but mentioned no attachment by mouthparts. Smart's (1934) account differs from mine only in that he did not mention exactly where the anal proleg attached in relation to the thoracic proleg. During looping, *Simulium vittatum* larvae attach the anal proleg between the thoracic proleg and head, while both are attached to the pad.

Figure 6. Diagram of larva looping laterally, dorsal view.

a. Larva spinning silk onto substrate.

b. Larva attaching anal proleg, resulting in poor orientation of its anal proleg. The anal proleg will have to twist to allow the larva's body to trail downstream.

c. Intermediate step between "a" and "d." Larva swinging abdomen to its opposite side.

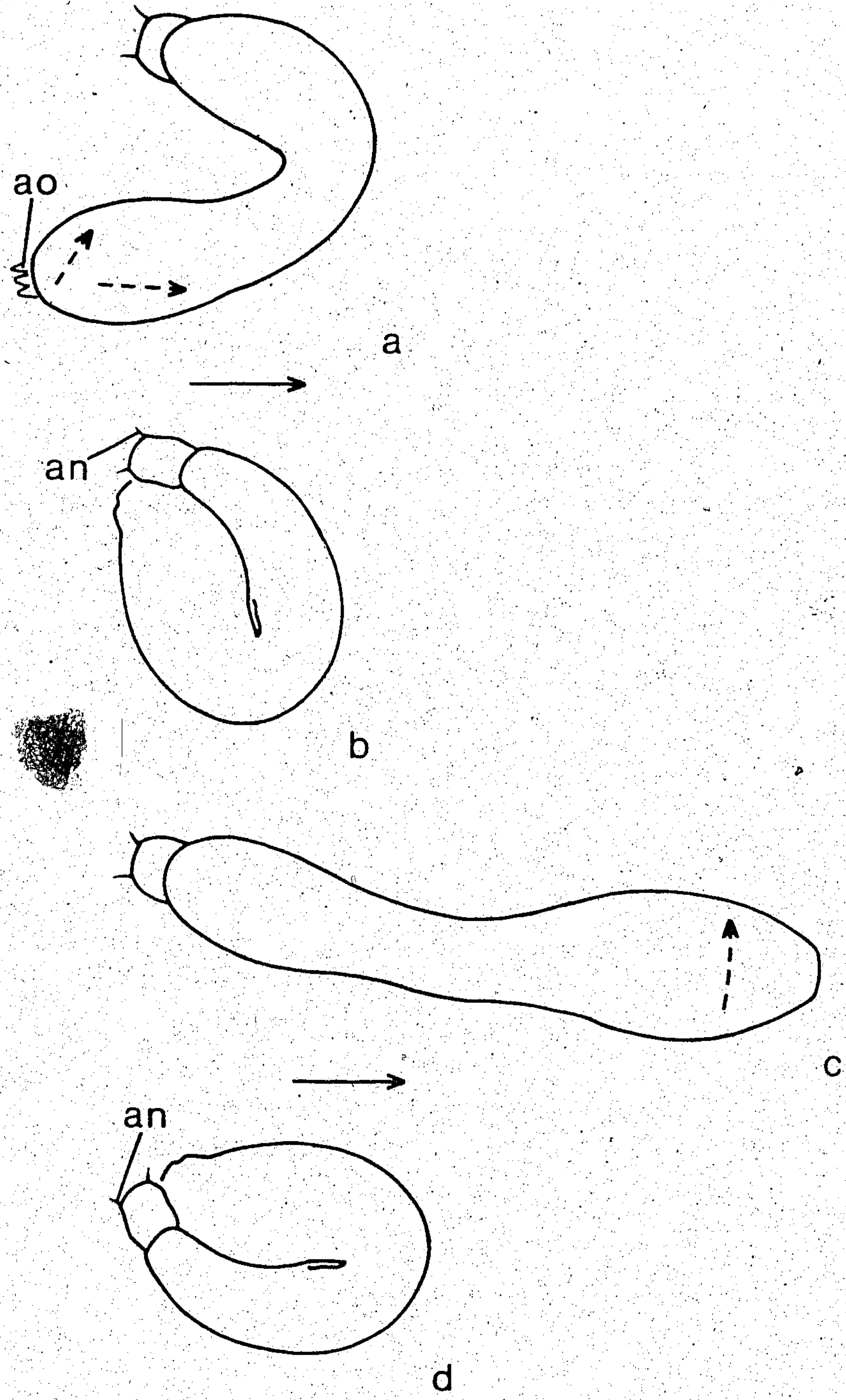
d. Larva attaching anal proleg, resulting in good orientation of its anal proleg.

Broken arrows point where abdomens move when larvae bring anal proleg from old pads of silk to new ones.

Solid arrows point downstream.

an- antenna,

ao- lobe of anal organ.



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Headlee (1906) commented (confirmed here) that larvae retract their anal organ during looping. This probably protects the anal organ and certainly allows the attaching anal proleg to fit close against the venter of the head capsule. My results also confirm Smart's (1934) observation that the centre of the anal proleg retracts as the whole proleg detaches. This has implications (Chapter 5) for understanding how the anal proleg attaches.

Larvae keep their cephalic fans closed while looping. They also keep them closed while spinning cocoons (pers. obs.). Chance (1970) observed *Simulium vittatum* larvae spinning silk with their cephalic fans extended, but my observations suggest either she was mistaken or she observed a rare event. My results support Chance's (1970) observation that mandibles and maxillae move rapidly during spinning of silk pads. Dorsal jerks of the head on release from pads probably result from release of pressure of the abdomen on the head and thorax.

Disagreements among previous accounts, and between my account and previous ones, could be due to different ways of looping between species of black-flies, but this is unlikely. Both Puri (1925) and Rubtzov (1962) based their accounts on observations of larvae belonging to more than one species in Simuliidae. Chance (1970) wrote about looping after studying live larvae in at least five species, including the fanless *Twinnia biclavata* Shewell [=*Twinnia nova* (Dyar and Shannon) (Wood, 1978)] and *Simulium vittatum*.

None of these authors mentioned interspecific differences in methods of looping. These differences are more likely due to their lack of equipment that could record behaviour of larvae in ventral view at some magnification.

The "D" shape of the attached anal circlet of hooks has implications for the study of how the anal sclerite aids attachment (Chapter 5), and for the interpretation of features of pads of silk larvae leave behind (Chapter 6).

Larvae usually have their anal prolegs attached as in Figure 3, the anal sclerite upstream of the rest of the anal proleg. This allows their bodies to trail downstream from the attachment. This orientation results from looping upstream of where the anal proleg is attached (Figure 4). Larvae spin silk and attach mouthparts upstream of the thoracic proleg, and the anal proleg attaches to the silk in between. When larvae loop downstream of where the anal proleg is attached the anal proleg attaches to the new pad of silk opposite to usual orientation (Figure 5). Larvae in this orientation to the current probably risk detachment or injury because the posteroventral position of the anal proleg (Chapter 5) will not allow larvae to lie on their backs.

Larvae can filter-feed from attachments produced from looping laterally, but larvae expose their bodies to more of the current than when looping upstream, as a comparison of Figure 6a with Figure 4a shows. Also, swinging the abdomen downstream as in Figure 6c seems a risk to attachment. Both problems, first, more exposure to the current, and second, more risk of detachment from swinging the abdomen downstream, probably become more acute at higher velocities of current.

Preferred larval orientation, together with a minimizing of exposure to the current could predispose larvae to looping upstream rather than in other directions. Lewis and Bennett (1975), after finding uniform distribution of black-fly larvae along streams they studied, reasoned that larvae actively migrate upstream, counterbalancing the effect of drift. If larvae are predisposed as described above, to looping upstream, this could help to impel upstream migration.

As Smart (1934) first hinted, larvae bend their bodies laterally through all of looping: spinning new pads and attaching the anal proleg to them; looping upstream, downstream, and laterally (Figures 2 to 6). Larvae appear unable to bend their bodies far dorsoventrally, even during capture (pers. obs.). Rubtzov (1962) showed in a diagram a larva bending dorso-ventrally as it looped downstream. But larvae unable to bend laterally could not loop upstream or even laterally without first turning themselves about. The current would thwart attempts by those larvae to rotate themselves about the anal proleg. Also, only by bending laterally can larvae attach the anal proleg between the attached head and thoracic proleg.

Bending laterally during looping, browsing (Craig, 1977; Serra-Tosio, 1967), and protecting themselves (Chance, 1977; Grenier, 1949), larvae keep their bodies close to the substrate, where the current is slowest (Chance, 1977).

2.4.1 Origin of lateral bending

I suggest that lateral bending during locomotion is the rule among larvae in Culicomorpha, a taxon of flies that includes Simuliidae (Figure 42; Hennig, 1973).

Nachtigall (1965; 1974a) illustrated ceratopogonid, chironomid, culicid, and chaoborid larvae all swimming by lateral bendings of their bodies. Dixid larvae also bend laterally when swimming (Nachtigall, 1965; 1974b). Except for *Ceratopogon* (Ceratopogonidae), all larvae seemed at one point during their bendings to bring the end of the abdomen to the head (Nachtigall, 1965; 1974b). *Atrichopogon* and *Forcipomyia*, more primitive ceratopogonids (Hennig, 1973), crawl about as larvae by bending their bodies sideways, gripping the substrate with their anal prolegs, then straightening again (Saunders, 1925), similar to simuliid looping. Thaumaleid larvae move by lateral bending (Saunders, 1923; Theinemann, 1909).

Dixid and thaumaleid larvae live at the interface of water and land, breathing the air above the water and grasping the substrate below the water (Hennig, 1973). In these habitats, films of water covering solid substrates, perhaps ancestral for culicomorphan larvae, dorso-ventral bendings of the body would require work against surface tension of the water. Ancestral locomotion by lateral bending in films of water could have preadapted descendants (Simuliidae) for locomotion by lateral bending in slow water close to the substrate in rivers and streams.

3. Spinning and manipulation of silk by mouthparts

3.1 Introduction

Opinions prevailed that a black-fly larva spins flat filaments of silk because its salivarium is a slit (Debot, 1932), that a larva grasps its silk with hooks on its labropalatum (Hora, 1930; Grenier, 1949), and that it cuts its silk with its mental teeth alone (Wood *et al.*, 1963; Crosskey, 1973). I propose alternative mechanisms in this chapter, and support alternate mechanisms others have lately proposed of how a black-fly larva spins out, grasps, and cuts off its silk. I use "spin" in the same sense as Rudall and Kenchington (1971), that is, the process of extrusion of silk, with "...no obvious twisting of filaments...."

A black-fly larva spins silk with its labiohypopharynx, and through its salivary meatus (Craig, 1977; Grenier, 1949). The labiohypopharynx bears many brushes (Chance, 1970; Couvert, 1970; Grenier, 1949; Puri, 1925), and a pair of short labial palpi (Craig, 1977).

Debot (1932) proposed that muscles of the press (part of the common salivary duct) pump silk forward from the labial glands through the salivarium and out, where hooks of the thoracic proleg grasp the silk and pull it out still farther. He also implied that filaments of silk flatten as they pass through the salivarium. I propose instead that spinning by maxillae and anterodorsal movement of the salivary meatus produce wide, flat filaments of silk.

Couvert (1970) illustrated maxillary muscles and external stucture most clearly. Chance (1970) and Craig (1977) also described external maxillary structures, including several brushes, a sclerotized adoral lobulate area, and a row of lacinial spines. Craig and Borkent (1980) gave an aboral view of the whole maxilla, and described maxillary sensilla, and those of the palpus, in detail. Chance (1970) and Cook (1949) also described maxillary muscles. Chance (1970) reported that maxillae move as larvae spin silk, and S.J. McBride (in Riley, 1870) wrote that before attaching the rear proleg a larva works its maxillae against the substrate. I propose that aboral and ventral maxillary brushes and lacinial spines pull silk from the salivary meatus and help to apply that silk to the substrate.

A black-fly larva can apply some silk to the substrate and, letting out a line of silk, trail downstream from this anchorage. Tarshis and Neil (1970) reviewed this subject. Larvae can climb back up these anchored lines using their prolegs and mouthparts (Chance, 1970; Jobbins-Pomeroy, 1916; Puri, 1925; Wu, 1931). Hora (1930) and Grenier (1949) suggested that larvae grasp such anchored lines (and pads of silk) with bristles of their labropalatal brushes.

On the other hand, *Simulium vittatum* larvae can grasp anchored lines with their mouthparts and support themselves even in mid air, although bristles of their labropalatal brushes are ". . .not structurally suited . . . ." (Chance, 1970, p. 270) for grasping anchored lines. The labropalatum includes the cephalic fans (lateral palatal brushes) and the area between the stems of cephalic fans extending posteriorly to the cibarium (Harbach and Knight, 1980). Chance (1970) illustrated labropalata of *S. vittatum* larvae in anterior and lateral views. This chapter includes evidence corroborating Chance's observations of the weakness of labropalatal bristles and supporting her conclusion that black-fly larvae do not grasp their anchored lines with labropalatal brushes.

Craig (1977) found that larvae climbing their anchored lines of silk moved their mandibles rapidly and repetitively. Chance (1970) reported that larvae rarely move their mandibles as they spin their cocoons, but according to Craig (1977), they do. Craig (1977) studied the structure of simuliid larval mandibles in detail. I present further evidence that mandibular teeth grasp anchored lines of silk.

The mentum, probably formed from two folds in the ventral cuticle (Anthon, 1943) of the labium (Craig, 1969), protects the rest of the labium of dipteran larvae that have complete head capsules (Anthon, 1943). Wood *et al.* (1963) and Crosskey (1973) suggested that mental teeth could cut filaments of silk. The following includes a detailed study of the external structure of the mentum, and gives further evidence of its role in cutting filaments of silk.

Craig (1977) moved the mandibles of dissected head capsules to make contact with the mentum, and suggested that mandibular and mental teeth of *Simulium oviceps* are so well coadapted, that when they meet, they probably cut filaments of silk that the larva produces. I will extend Craig's observations to show that mandibular teeth of more typical black-fly larvae swing past mental teeth closely, and that this shearing probably helps to cut filaments of silk.

3.2 Materials and methods

Rothfels and Featherston (1981) reported that larvae from the Sturgeon River (about 25 km NW of Edmonton, Alberta) belong to the IS-7 sibling of *Simulium vittatum*. Larvae and water from that site were transported for about an hour in buckets, then transferred to glass rearing tanks (about 25 x 20 x 40 cm). Air bubbled from the bottom of each tank circulated the water, and a water bath around the tanks kept the temperature between 1° and 4°C. Water in the buckets during transport was neither circulated nor cooled. Larvae were kept in the tanks for up to six months. Growth of larvae reared for over a month in the tanks probably was inhibited because the rearing water was not changed and excretory material probably built up. Unless indicated, figures and dimensions are of last-instar larvae. Developmental stage was determined from criteria in Ross (1979). These determinations could be an instar in error since the population Ross (1979) studied was probably cytologically different from the Sturgeon River population.

Larvae killed for study of structure in 95% ethanol retracted their anal papillae but protracted their prolegs and mouthparts. Larvae killed by freezing in a drop of water (in a Petri dish or on a sheet of stainless steel chilled by contact with dry ice) strongly retracted their mouthparts, anal papillae, and prolegs as they thawed. This was probably because ice crystals lysed cells and calcium ions diffused from the haemolymph to muscle filaments, resulting in contraction. Larvae killed in boiling distilled water died relaxed. A larva killed in a 3:1 mixture of 95% ethanol and glacial acetic acid was transferred to distilled water where its maxillae and labiohypopharynx were removed through the foramen magnum. The head capsule remaining was cleared in boiling lactic acid, and the mandibles were pushed toward the mentum (Craig, 1977).

Two larvae killed in boiling water were soaked in solutions of increasing ethanol concentration up to 95% ethanol. One minute's soaking in 95% ethanol saturated with quinone toughened large expanses of cuticle on those two specimens. This is a new method aimed at lessening wrinkling of cuticle due to shrinkage of soft tissue (Cohen, 1979) during critical point drying. Longer exposure to quinone left cuticle brittle and subject to buckling and cracking.

Larvae killed by immersion in boiling distilled water or by freezing were soaked overnight in cold 4% glutaraldehyde in sodium phosphate buffer (pH 7.2), rinsed in buffer and distilled water, then dehydrated in acidified 2,2-dimethoxypropane (DMP) (Muller and Jacks, 1975), then critical-point dried in preparation for examination by scanning electron microscope. The labiohypopharynx of a larva killed in boiling distilled water was removed in distilled water, dehydrated in acidified DMP (Bjerke *et al.*, 1979), and critical-point dried. Larvae in 95% ethanol were critical-point dried directly. Specimens were sputter-coated with gold and examined either with a Cambridge S4 or S150 scanning electron microscope at various accelerating voltages. Photomicrographs were made either on Kodak Plus-X or Tri-X Professional 120 roll film.

3.3 Terms

Harbach and Knight's (1980) standard terms for mosquito structures, possible homologies, and terms of other authors influenced my choices of term (Appendix Tables A-1 to A-8). I adopted Craig's (1977) terms for structures of larval mandibles.

Rubtzov (1959-1964) named the sensilla of the labial palpus by their relative positions, but I tried to homologize these sensilla with those of mosquito larvae, and to follow Harbach and Knight's (1980) system of numbering.

Lack of sockets for sensilla S1, pairing of S2 and S3, and the medial positions of sensilla S4 and S5 guided my choices of possible homology. Craig (1977) showed clearly that the sensillum called "S1" here has no socket. Homologies proposed here between black-fly and mosquito larval labial palpal sensilla have only weak support. Especially tenuous is the homology between the mid-dorsal sensillum on the simuliid larval labial palpus, and sensillum S1, dorsal to the palpus of mosquito larvae (Harbach and Knight, 1980).

On the other hand, the number, shape, and position of these sensilla appears stable among black-fly larvae. Couvert (1967, 1970), Craig (1977), Crosby (1974), and Rubtzov (1959-1964) all found five sensilla similarly arranged on the labial palpus. Chance (1970) reported more sensilla, but was probably counting other projections on the palpus.

3.4 Results

3.4.1 Labiohypopharynx

The salivary meatus is a slit across the labiohypopharynx through which silk (si) emerges (Figure 7). The transverse part of the meatus, between the anterolateral projections (hp) of the hypopharynx, is about 80 um wide (Figure 7), and the whole slit is about 280 um wide (Figure 13). The labiohypopharynx and the mentum (m) below it widen posteriorly together (Figure 13), so that the meatus (x) and corresponding edges of the mentum are nearly parallel (Figure 12a).

Above the meatus, the hypopharyngeal brush has three rows (r1-r3) of bristles (Figure 7). Row r1 extends between the two anterolateral projections (hp) of the hypopharynx, about 70 um apart. Row r2, ventral to r1, is about 100 um long. Row r3 borders the salivary meatus for almost its whole width. All bristles of row r1 are branched at their tips, some bristles in row r2 are branched, but those of row r3 are simple. Row r3 is at least three bristles wide laterally on the labiohypopharynx, but otherwise, each hypopharyngeal row is one bristle wide.

Below the salivary meatus, the dorsal premental brush (dp), with its double row of bristles (Figure 8), extends as far laterally as row r3 of the hypopharyngeal brush extends (Figure 7).

Anteroventral to the dorsal brush, and medial on the prementum, lie the short labial palpi (Figure 7). Sensillum S1 of the labial palpus (Figure 8) is conical and unsocketed, sensilla S2 and S5 is conical and socketed, and sensillum S3 is cylindrical and socketed, with a conical tip of perhaps six appressed finger-like lobes. Sensillum S4 is socketed and shaped like a cone squashed from one side. Medial to sensillum S5 are two rows of projections corresponding to Harbach and Knight's (1980) "sixth sensillum" on mosquito larvae. Projections dorsal and

Figure 7. *Simulium vittatum* larva. Labiohypopharynx, left side, anterior view.
Scale=20 um.

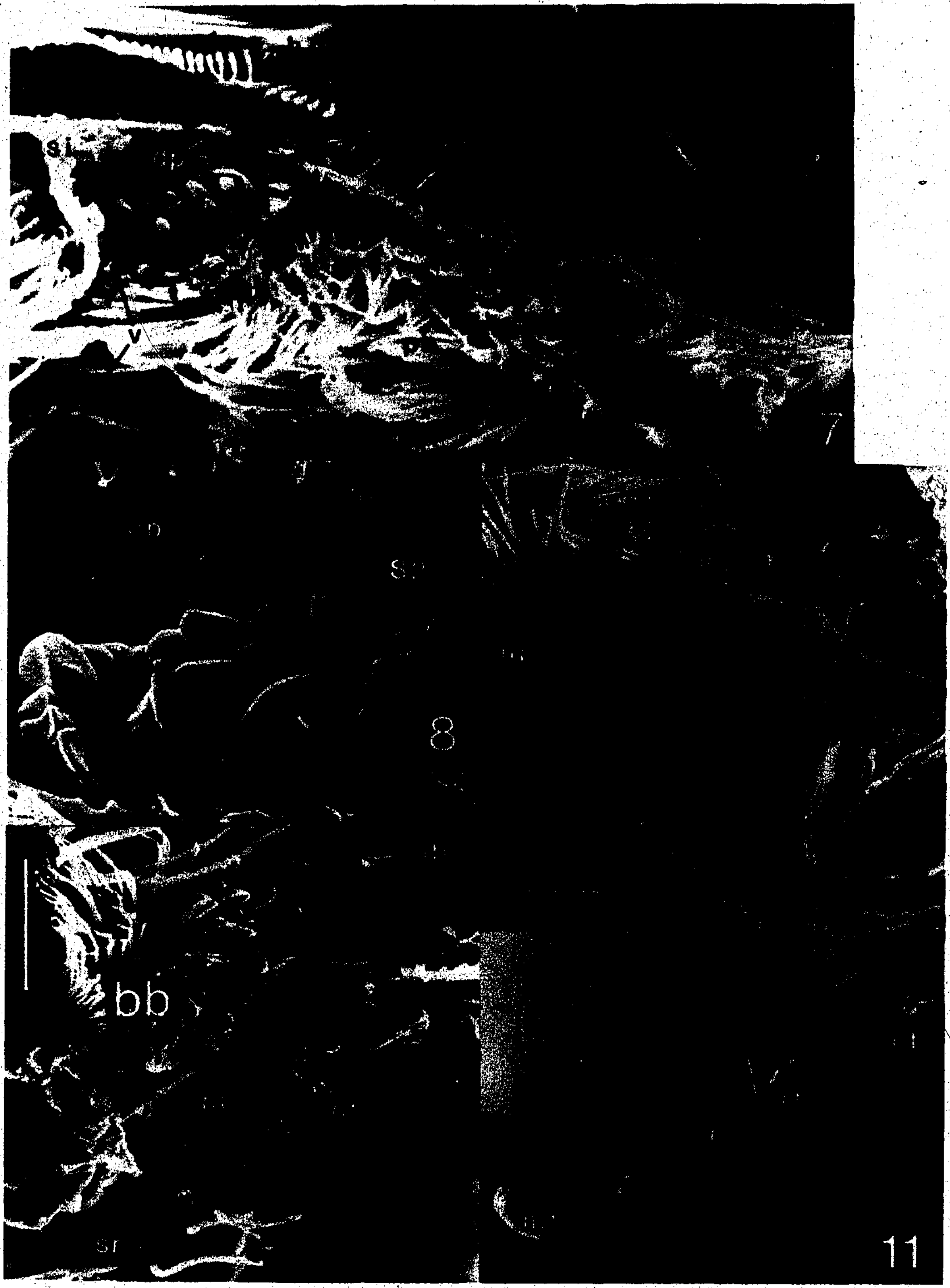
Figure 8. Left labial palpus, anterior view.
Scale=4 um.

Figure 9. Right maxilla reflected from labiohypopharynx, anterior view.
Scale=40 um.

Figure 10. Labiohypopharynx and right maxilla spinning out silk, ventral view.
Scale=40 um.

Figure 11. Mental teeth, right side, anterodorsal (internal) view.
Scale=10 um.

ab- apical brush of mandible,
bb- aboral maxillary brush,
ct- corner tooth of mentum,
dp- dorsal premental brush,
ds- apicodorsal maxillary sensillum,
fl- flange of mental tooth,
hp- anterolateral projection of hypopharynx,
hx- hypopharynx,
it3- third intermediate tooth of mentum,
la- lacinia,
lp- labial palpus,
mt- median tooth of mentum,
pb- lateral premental brush,
r1 through r3- first through third rows of hypopharyngeal brush,
si- silk,
sr- lateral serration of mentum,
S1 through S5- sensilla 1 through 5 of labial palpus,
tl- lacinial teeth,
v- ventral premental brush,
va- ventral adoral maxillary brush,
vm- ventromentum,
x- salivary meatus.


si
v
sp
7
8
bb
sr
11

medial to sensillum S4 lack obvious pores and sockets. Each palpus has a ventral and a lateral plate of cuticle (Figure 8).

The ventral premental brush (v) borders the palpi ventrally (Figure 7). Lateral premental brushes (pb) border on the ventral brush and the palpi medially, and extend almost to the ends of the salivary meatus (Figures 7 and 9). Lateral brushes are dense and extensive (Figure 7). Their bristles are about twice as long as those of the ventral brush, and are less branched (Figure 7). Bristles of the lateral premental brush (pb) lie between mental teeth in Figure 10.

3.4.2 Maxillae

Maxillae are dorsolateral to the salivary meatus (Figure 13). A triangular shield of cuticle on the lacinia (la) faces the side of the hypopharynx (hx, Figure 9), and wields on its apex and ventral edge eight teeth (tl, Figure 9). The ventral adoral maxillary brush (va), medial on the lacinia, has eight rows of fine bristles. The ventral aboral brush (vb), ventral and basal on the maxilla (Figure 13), has short, weak bristles pointing anteriorly. Bristles of the much larger aboral brush (bb) also point apically (Figures 9 and 13). The longest bristles of this brush are more apical; the shortest, basal (Figure 13).

Aboral maxillary brushes (bb) and lateral premental brushes (pb) overlap longitudinally (Figure 13). Silk (si) is caught on bristles of the aboral maxillary (bb) and the lateral premental brushes (pb) in Figure 10.

The aboral maxillary and lateral premental brushes are at the same level in Figure 10, but apart in Figure 13, so maxillae are able to move independently of the labiohypopharynx.

Tips of apicodorsal maxillary sensilla (ds) project ventral to mandibular teeth, and anterior to the mental teeth (Figures 10 and 16).

3.4.3 Mandibles

Although mandibles swing on condyles dorsal to maxillae, teeth of adducting mandibles meet and cross anterior to the salivary meatus. The apical tooth (at) of the mandible has two longitudinal grooves (gr), one adoral one aboral (Figure 15). A suture line extends from the ends of the aboral groove (Figure 15). Larval mandibles of other black-fly species have grooves in the apical tooth (Craig, 1977), so the apical mandibular tooth appears comprised of two fused teeth. The first outer tooth (ot1) touches the medial side of the third outer tooth (ot3, Figure 15). The second outer tooth (ot2), about 19 um long, has a basal spur (Figure 15). Mandibular teeth point ventrally and posteriorly, but outer teeth, especially the second (ot2), point more medially than the others (Figures 15 and 16).

Bristles of the apical mandibular brush (ab, Figure 15), arranged in rows and bent at their tips, point in the

Figure 12. Proposed mechanism of spinning of wide and narrow filaments of silk,

a. ventral view,

b. lateral view.

Arrows point anteriorly.

m- mentum,

si a- narrow filament of silk produced when salivary meatus moved posteroventrally,

si b- wide filament of silk produced when salivary meatus moved anterodorsally,

x- salivary meatus.

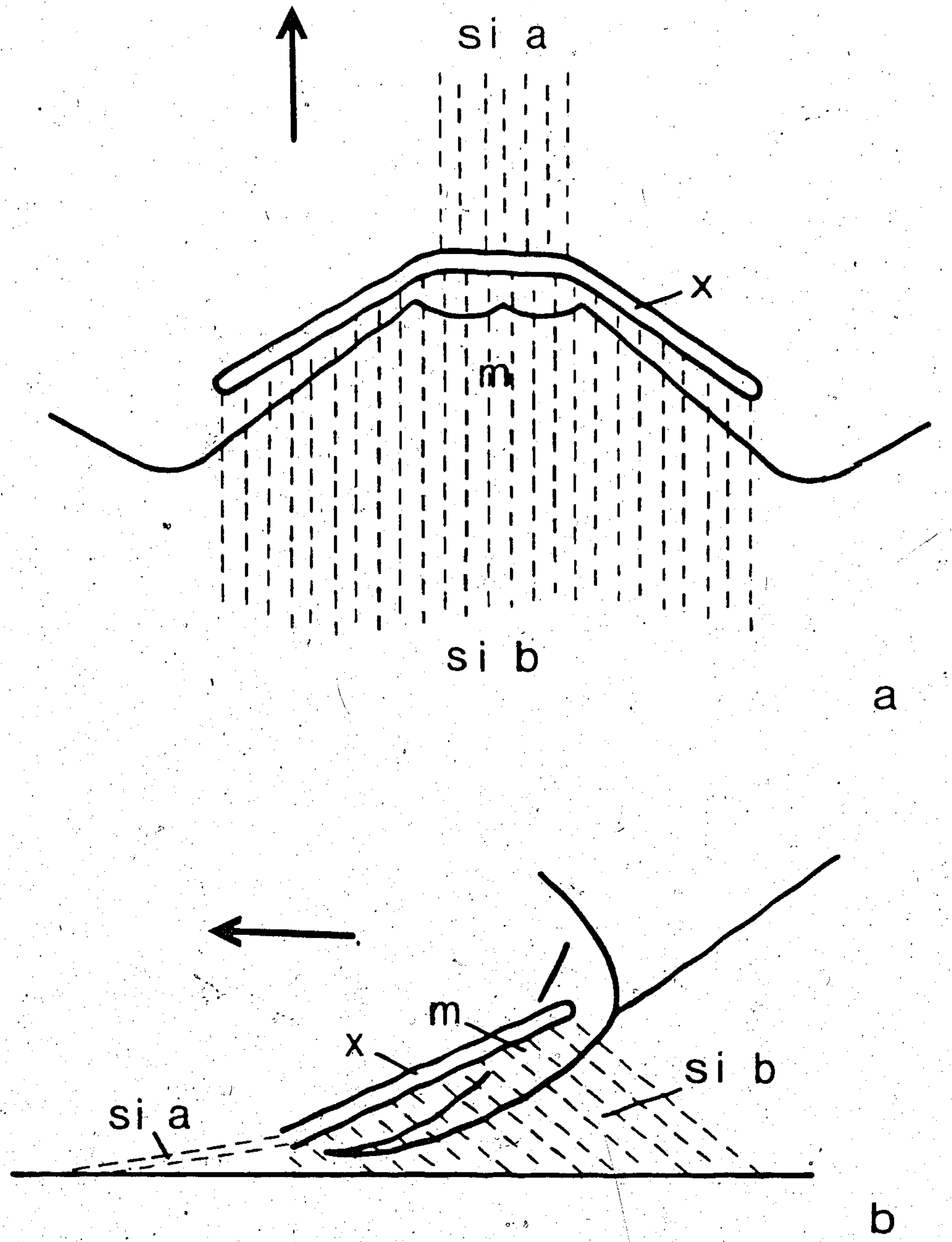


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Figure 13. *Simulium vittatum* larva. Fully protracted maxillae, ventral view.
Scale=200 um.

Figure 14. Mandibular teeth adducted to mental teeth, filament of silk cut, anterior view, left side.
Scale=10 um.

Figure 15. Apex of left mandible, median surface.
Scale=10 um.

Figure 16. Mandibular teeth adducted to mental teeth, ventral view.
Scale=20 um.

Figure 17. Mandibular teeth pushed to mental teeth after maxillae and labiohypopharynx removed, lateral view of right side.
Scale=20 um.

Figure 18. Sixth-instar larva. Bristles of labropalatal brushes, ventrolateral view.
Scale=4 um.

ab- apical brush of mandible,
at- apical tooth of mandible,
bb- aboral maxillary brush,
cb- covering brush of mandible,
ds- apicodorsal maxillary sensillum,
fl- flange of mental tooth,
gr- groove in apical tooth of mandible,
it1, it2- first and second intermediate mental teeth,
la- lacinia,
lt- lateral mental tooth,
m- mentum,
mb- median labropalatal brush,
mp- mid-labropalatal brush,
mt- median tooth of mentum,
ot1 through ot4- first through fourth outer teeth of mandible,
pat- preapical tooth of mandible,
pb- lateral premental brush,
si- silk,
sr- lateral serration of mentum,
st- spinose teeth of mandible,
tl- lacinial teeth,
va- ventral adoral maxillary brush,
vb- ventral aboral maxillary brush,
x- salivary meatus.


tl
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13


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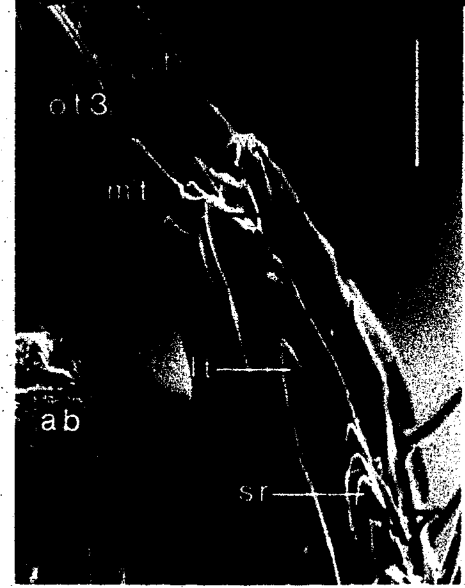
ot3
mt
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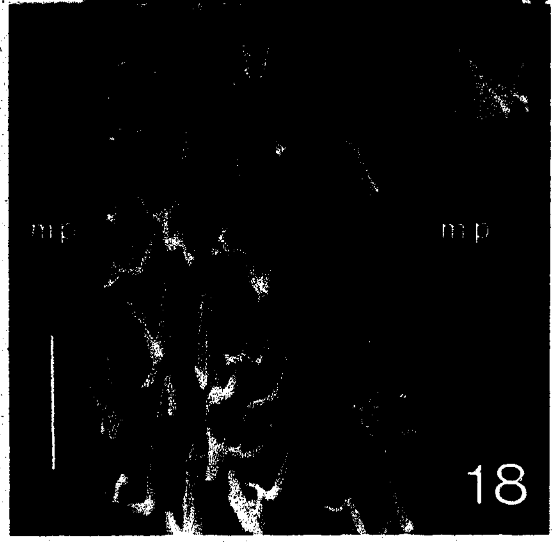
ab
sr


mp
mp
18

same direction as the fourth outer tooth (ot4). This brush (ab) cannot reach the mental teeth when the mandible is adducted (Figure 17).

The right mandible in Figure 16, its apical brush labelled "ab", is crossed behind the left mandible. Both were partially adducted. On one mandible examined, a bit of silk covered the tips of the fourth outer, the apical and the preapical teeth, and one of the mandibular serrations. The tip of the fourth outer tooth of another mandible examined was broken.

3.4.4 Labropalatum

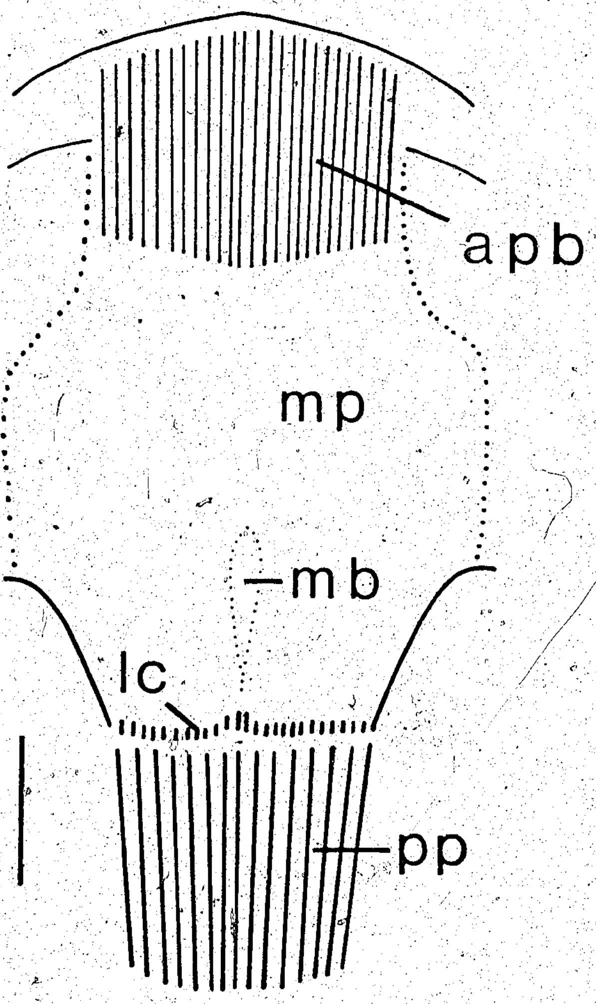
Since black-fly larvae are prognathous, the labropalatum is dorsal to the salivary meatus. The median labropalatal brush (mb) is posteromedial on the labropalatum (Figure 19). Bristles of the median brush emerge from raised bases, in single longitudinal rows of about eight bristles (mb, Figure 18). Bristles of the mid-labropalatal brush (mp, Figure 19) are in bunches of about ten bristles, arranged in two transverse rows of about five bristles each (Figure 18). Bristles of both brushes are thin and bent (Figure 18).

3.4.5 Mentum

The mentum is ventral to the labiohypopharynx. Mental teeth, well-braced posteriorly (Figure 17), point anterodorsally (Figures 11 and 17). The tip of each is

Figure 19. Diagram of labropalatal brushes, ventral view, based on SEM examination.
Scale=100 um.

apb- anterior palatal brush,
lc- labropalatal comb,
mb- median labropalatal brush,
mp- mid-labropalatal brush,
pp- posterior labropalatal brush.



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nipple-like (Figure 14), and a flange (fl) fringes the base anteriorly (Figure 11). Flanges are thin (Figures 14, 16 and 17) and serrated (Figure 11). Narrow notches separate neighboring flanges (Figure 11), but together they make a sinuous line (Figure 14). Lateral teeth (lt) have flanges, but lateral serrations (sr) do not (Figure 17). Since the mental teeth are excavated ventrally (Figures 14 and 17), the ventromentum (vm) is a ridge.

A first intermediate tooth (it1) of the mentum in Figure 17 is broken. On another specimen examined, a first intermediate tooth, the median tooth, and a corner tooth were broken.

Larvae killed by freezing adducted their mandibular teeth past their mental teeth, as in Figure 17, where the right mandible (its apical brush is labelled "ab") was pushed past the mental teeth. The apical mandibular tooth fits between the first and second intermediate mental teeth in Figures 14 and 16, and the fourth outer mandibular tooth fits between the median tooth of the mentum and the first intermediate mental tooth. The third outer mandibular tooth fits on the contra-lateral side of the median mental tooth.

3.5 Discussion

3.5.1 Spinning out filaments of silk

Black-fly larvae make flat ribbon-like filaments of silk (Debot, 1932; Grenier, 1949) probably by spinning with anterodorsal movements of their heads, and by drawing silk out with their maxillae. The internal shape of the salivarium is unable alone to flatten filaments of silk since larvae killed in ethanol die spewing out rounded filaments of silk (Craig and Borkent, 1980, their Figure 3) thicker than attachment pads (pers. obs.). When a larva spinning silk moves its head anteriorly or dorsally to where the filament is fixed, the pull of the silk over the snowplow-shaped salivary meatus (Figure 12) probably forces some silk out the sides of the meatus, stretching the silk filament into a wide ribbon. Anterior and dorsal movements of the head would in this way result in a filament of silk up to 280 um wide, the width of the salivary meatus.

Posterior and ventral movements of the head would force silk into the transverse part of the salivary meatus, and result in filaments of silk only 80um wide, or less. This narrow filament would be narrower than the toothed part of the mentum (100 um), and thus be easily cut there. The shape of the mentum corresponds to the shape of the salivary meatus and would help to support the prementum during spinning (Figure 12).

The hypopharyngeal brush is at least stiff enough to leave grooves in the silk filament (Craig, 1977). Since bristles of the lateral premental brush can penetrate silk (Figure 10), and since bristles of the dorsal premental and hypopharyngeal brushes are stouter and less branched, bristles of the hypopharyngeal and dorsal premental brushes, below and above the salivary meatus, probably penetrate silk and prevent it from retracting into the labiohypopharynx, as Grenier (1949) proposed.

During spinning, the labiohypopharynx moves frequently (Grenier, 1949). Ventral and lateral premental brushes would help to apply silk onto the substrate. Premental brushes also appear to keep the filament of silk from being caught on mental teeth while silk is being spun (Figure 10).

The maxillae and the labiohypopharynx can move independently, the lacinial shield sliding on (Figure 9) and off (Figure 13) the side of the hypopharynx. Since bristles of the aboral maxillary brush can penetrate silk, protracting maxillae could help to draw silk out of the sides of the salivary meatus. This could explain the observation in Chapter 2 of maxillae moving when larvae were applying silk to the substrate, and similar observations of Chance (1970) and McBride (in Riley, 1870). This could help also to widen filaments of silk. If the filament of silk is cut at the mentum, and does not retract into the salivary meatus, the lacinial spines could grasp the free end of the filament when the larva starts to spin silk again.

This study is the first to describe the ventral aboral brushes of the maxilla. Lateral premental brushes would probably take particles of food that these brushes gathered, but ventral aboral brushes more likely help spin silk out the sides of the salivary meatus. The ventral aboral brushes (vb) in Figure 13 are directly anterior to the lateral borders of the salivary meatus (x).

Sensilla of the labial palpi and the galeae (apicoventral and apicodorsal) could sense whether food or silk covered the substrate. Sensilla of maxillary palpi of larvae lacking cephalic fans probably touch the substrate (Craig and Borkent, 1980).

3.5.2 Grasping silk with mouthparts

Black-fly larvae can probably grasp pads and anchored lines of silk with their outer mandibular teeth.

Hora (1930) and Grenier (1949) thought that larvae grasp anchored lines with their median labropalatal brushes, but bristles of these brushes appear too weak to bear forces of drag on larvae attached only by anchored lines. Chance (1970) also found labropalatal bristles of *Simulium venustum* Say/*S. verecundum* Stone and Jamnback, *S. decorum* Walker, *Cnephia dacotensis* (Dyar and Shannon), *Twinnia biclavata* Shewell [=*Twinnia nova* (Dyar and Shannon) (Wood, 1978)], and *Prosimulium travisi* Stone larvae too weak for grasping anchored lines of silk. On the other hand, median brushes are so dense (Grenier, 1949), that if they worked together, bristles could perhaps share large loads without damage.

Peterson's (1977) figure suggested that the posterior comb (pc) of *Simulium vittatum* larvae is part of the median labropalatal brush (mb, Figure 19), but results presented in this chapter agree with those of Chance (1970) who found the median brush and the posterior comb are separate.

Larvae adduct their cephalic fans before spinning or grasping silk with their mouthparts (Chapter 2). Adducted cephalic fans cover the posterior half of the labropalatum (Craig, 1977, his Figures 3 and 4). Since the median brush is on the posterior half of the labropalatum (Figure 19), cephalic fans probably cover that brush when larvae are holding onto lines and pads of silk with their mouthparts.

Because the bristles of labropalatal brushes are weak, and because they are covered by the cephalic fans when larvae spin and grasp silk with their mouthparts, black-fly larvae probably grasp pads and anchored lines of silk with other mouthparts. The labiohypopharynx probably does not grip anchored lines because all of its bristles point anteriorly, out from the salivary meatus. If the press in the common salivary duct restricted the flow of silk, this would only make the line thinner. Mental teeth also point anteriorly, the direction that anchored lines are let out. Chance (1970) suggested that mandibles and maxillae hold anchored lines, but the only strong maxillary structures are the lacinial teeth, and these point anteriorly.

Since mandibles cross when adducted at the same time, and since outer teeth point medially, the outer teeth must meet at some point anterior to the salivary meatus, and thus perhaps grasp anchored lines of silk. This would explain Craig's (1977) observations of larvae adducting their mandibles while climbing their anchored lines. Silk found on mandibular teeth also suggests that these teeth handle silk. On the other hand, if outer teeth brake anchored lines of silk, the lines could damage covering brushes of the mandible (cb, Figures 15 and 16) lying over those teeth. Because they swing through wide arcs, mandibles would be the mouthparts best suited for gathering the anchored line while the thoracic proleg held it, and the apical mandibular sensilla that Craig (1977) mentioned could help larvae to know when their mandibles had found an anchored line.

3.5.3 Cutting filaments of silk

Black-fly larvae probably cut filaments of silk with their mandibular and mental teeth.

The cut strand of silk (si) behind the mentum in Figure 14 supports the suggestions of Crosskey (1973) and Wood *et al.* (1963) that mental teeth cut filaments of silk. Observations here suggest that the sharp, serrated flanges of mental teeth aid the cutting of silk. Although nipple-like, tips of mental teeth are probably not sensory since D.A. Craig (pers. comm.) found neurons extending to the [illegible]choid mental sensilla but not to the mental teeth.

Mental teeth and labial brushes appear to clean each other (Figure 10) of food or silk. Labial brushes probably clean the mental teeth as the prementum is protracted, and mental teeth probably clean labial brushes as the prementum is retracted.

Since they barely reach them, bristles of apical mandibular brushes probably do not clean mental teeth. Larvae could instead browse the substrate with their apical brushes (Craig, 1977; Ross and Craig, 1979). Scrubbing the substrate and gathering particles dislodged by mandibular teeth would suit the bent tips of these bristles. An apical brush could also clean the oral side of a mandible crossed over it.

Mandibular teeth swing past mental teeth, meshing with them. Larvae could break mandibular and mental teeth while scraping the bottom, but poor meshings of teeth at other positions along the mentum could also explain the broken teeth observed. When at the mentum, mandibular teeth point in the opposite direction to mental teeth (Figure 17). Meshing of mandibular and mental teeth and cutting of filaments of silk on mental teeth imply that mandibular and mental teeth shear filaments of silk between them. Mandibular and mental teeth would perforate filaments of silk and the mandibles would help to stretch silk over the sharp flanges of mental teeth.

The relative positions of mandibular and mental teeth in Figures 16 and 14 are almost the same as Craig (1977, his

Figure 39) found for the left mandible of *S. tahitiense*. Couvert (1970) shows a mandible swinging near contra-lateral mental teeth. If not an artefact, this mandibular position could allow preapical (pat) and spinose teeth (st, Figure 15) to swing past mental teeth, perhaps when mandibles adduct one at a time. On the other hand, Craig (1977) showed that spinose teeth probably clean maxillary brushes, and oppose the lobulate area of the maxilla.

The shapes of mental teeth (*e. g.* Crosskey, 1969; Peterson, 1970) and mandibular teeth (*e. g.* Crosskey, 1969) are taxonomically useful. If these teeth mesh together, differences of mandibular shape should correlate with differences of mental shape. Perusal of Crosskey's (1969) illustrations of mandibular and mental teeth lend tentative support to this view. On the other hand, shapes of mandibular and mental teeth can vary intraspecifically (Couvert, 1967), perhaps making these correlations difficult to establish.

Opposition of mandible and mentum is probably widespread among eucephalous dipteran larvae. Mandibular teeth of *Chironomus plumosus* (Chironomidae) larvae swing past mental teeth and collect detritus (Gouin, 1957), and Darby (1962) hinted that *Cricotopus* (Chironomidae) larvae grated filaments of algae between mandibular and mental teeth. Mandibles of chironomid and culicid larvae swing obliquely like those of simuliid larvae (Cook, 1949), and mandibles of thaumaleid (Schremmer, 1951; quoted in Hennig,

1973) and ceratopogonid (Saunders, 1924, 1925; Lawson, 1951) larvae swing vertically. Larvae in all of these families could cut filaments of algae between mandibular and mental teeth. Chironomid larvae could also cut filaments of their own silk in this way.

0.0.1 Further work

Inferences in this study about how larvae spin out their silk, grasp onto it, and cut it off were based on shapes and relative positions of structures. Direct observations would test these inferences, and high-speed cinemacrographic records of larvae spinning pads and anchored lines of silk could provide these observations.

4. Structure and function of the thoracic proleg

4.1 Introduction

With its thoracic proleg, a black-fly larva can pull silk (Chance, 1970; Planchon, 1844) from its labiohypopharynx (Grenier, 1949), or attach itself to a pad of silk (Chance, 1970; Grenier, 1949; Smart, 1934), or hold onto an anchored line of silk (Puri, 1925; Hora, 1930; Grenier, 1949).

This proleg, an unpaired mid-ventral extension of the prothoracic segment (Grenier, 1949; Hinton, 1955; Puri, 1925), bears an apical circlet of hooks that grasp the silk (Grenier, 1949). Proximal to the circlet (Grenier, 1949) are a pair of lateral plates [=lateral sclerites (Crosby, 1974; Wood *et al.*, 1963)], with bristles on the distal edges (Grenier, 1949; Hora, 1930; Puri, 1925). Taxonomists describe differences in size and shape of these plates and sometimes use these differences as key characters (*e. g.*, Peterson, 1970).

Larvae can protract the distal article of the proleg to reach the mouthparts (Hora, 1930; Planchon, 1844; Tonnoir, 1923), or retract it below the level of the distal edge of the inflected proximal article (Puri, 1925, his Figure 2). Hooks, pointing radially, are pulled out of silk when a larva retracts the apex of the proleg, and are forced into silk when a larva allows hydrostatic pressure to protract and expand the apex (Grenier, 1949).

Since Grenier's study, morphologists have largely ignored the thoracic proleg, but there are good reasons for further study. For example, Grenier (1949) mentioned two gaps in the circlets of hooks, but no one has yet illustrated them. Also, no one has yet proposed a function for the lateral plates and their fringing bristles, other than as giving support to the distal article (Hora, 1930). Further, while anal prolegs of black-fly larvae are well-supplied with external mechanoreceptive sensilla (Grenier, 1949; Rubtzov, 1959-1964), no one has described sensilla from the thoracic proleg of any black-fly larva.

This study of the external structure of thoracic prolegs of *Simulium vittatum* larvae revealed:

1. that bristles of lateral plates could help larvae detach their thoracic hooks from silk, and
2. a pair of campaniform sensilla on the posterior surface, just outside of the circlet that could inform the larva of tension in the cuticle when the hooks are attached to silk or when the apex of the proleg is retracted.

4.2 Materials and methods

Materials and methods were the same as those in Chapter 3.

4.3 Results

Posterior (pg) and anterior gaps (ag) (Figures 20 and 21) in the circlet of hooks of the thoracic proleg split the circlet in two (Figure 24). While tines of hooks point radially, rows of hooks cross the circlet (from inside to outside) obliquely (Figure 21). Anterior rows slant away from the anterior gap, and posterior rows in their turn slant toward the posterior gap, that is, the outer hooks of rows beside the posterior gap (pg, Figure 20) are closest in their rows to the gap, but the outer hooks of rows beside the anterior gap (ag, Figure 21) are farthest from the gap. The first two or three rows to either side of a gap have fewer hooks than do the other rows (Figures 20 and 21).

Hooks of the thoracic proleg have neither tubercles nor serrations (Figure 20): they are smooth. Bases (ba) of hooks are raised above the level of cuticle that supports and connects them (Figure 20).

Lateral plates (lp) are less flexible than the surrounding cuticle (Figures 21 and 23). Bristles (bi) arise stiff and straight from the plates in groups of two to four (Figures 21, 22 and 23), and are inclined somewhat medially (Figure 23). About eighteen rows of hooks on a proleg are adjacent to bristles of lateral sclerites. The bristles lie between the silk and the bases of hooks when hooks are protracted into silk (Figure 22), but continue to point distally when hooks are retracted (Figure 23).

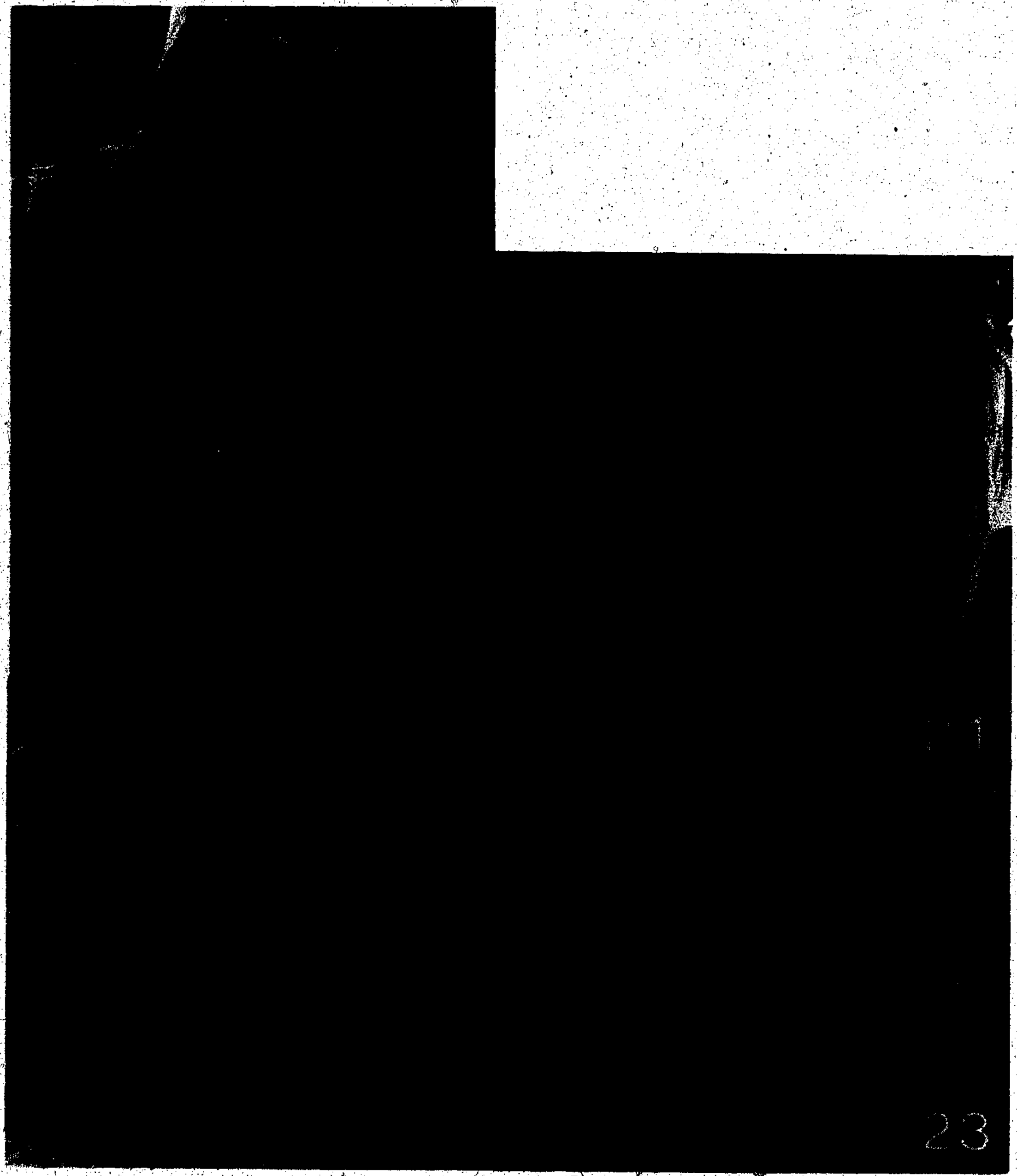
Figure 20. *Simulium vittatum* larva. Thoracic proleg, distal article, posterior view. Scale=10 um.

Figure 21. Thoracic proleg, distal article, anterior view. Scale=20 um.

Figure 22. Fifth-instar larva, thoracic proleg, distal article, postero-lateral view, right side. Scale=20 um.

Figure 23. Thoracic proleg, apex retracted, anterior view. Scale=20 um.

ag- anterior gap in circlet of hooks,
bi- bristle of lateral plate,
cs- campaniform sensillum,
pg- posterior gap in circlet of hooks,
pl- lateral plate,
si- silk.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On each side of the posterior midline, halfway to the base of the posterior-most bristle, is a campaniform sensillum (cs, Figure 20), each with a sharp-edged socket (Figures 20 and 22). These sensilla lie in transverse depressions in the cuticle (Figures 20 and 22) and, when a fold of cuticle covers its distal side, can appear "D"-shaped (Figure 22) rather than circular or oval (Figure 20).

4.4 Discussion

While hooks of anal prolegs have secondary tubercles (Figure 28), hooks of thoracic prolegs are smooth (Figure 22), and should move through silk easily. Unimpeded retraction from sticky (Chance, 1970) fresh silk should save larvae energy, because thoracic hooks grasp only fresh silk.

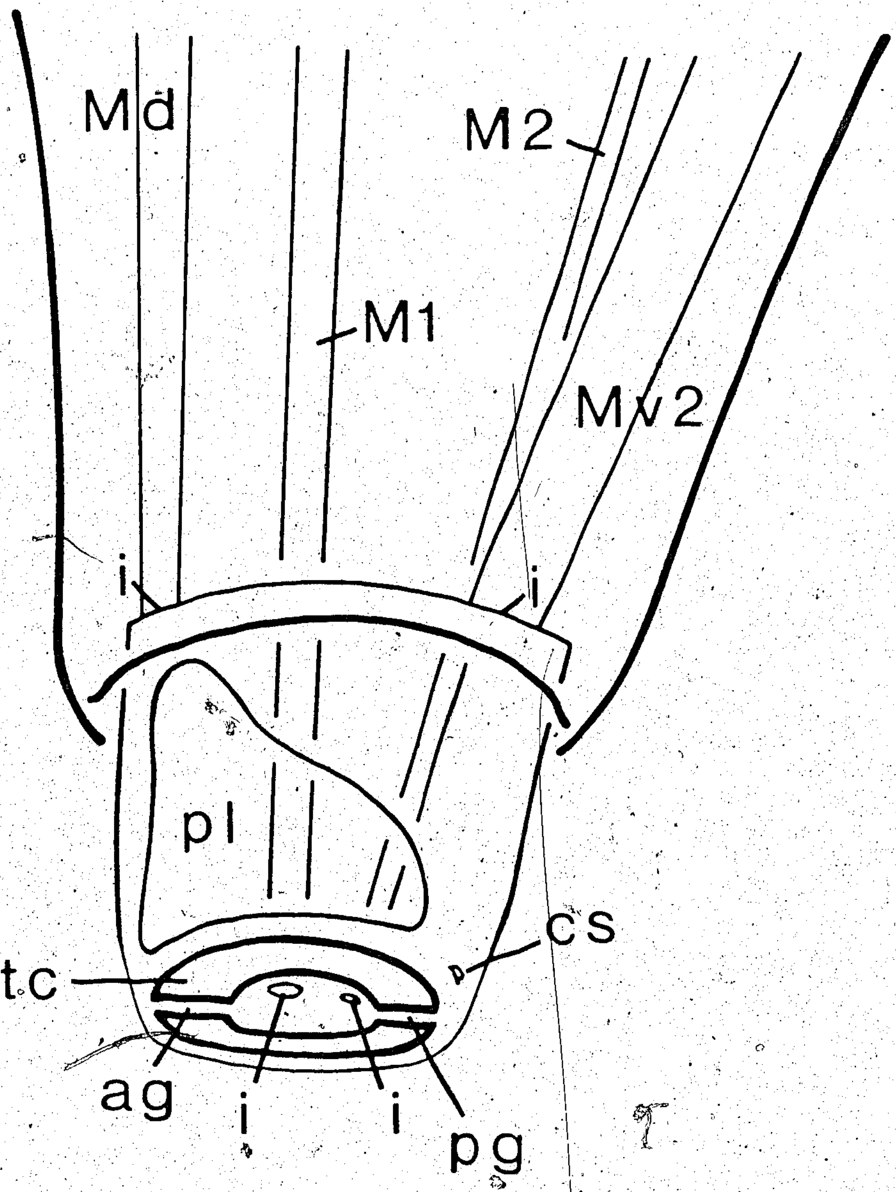
Bristles of lateral plates could also save larvae energy by holding elastic fresh silk down while hooks retract. Pull of muscles Md and Mv2 (Figure 24), rigidity of lateral plates, and push of surrounding reflexed cuticle of the proximal article (Figure 21) would help to keep the distal article cylindrical. Lateral plates, part of this cylinder, cause the bristles they support to point distally. When larvae protract their thoracic circlets during attachment to silk, bristles would lie down among lateral hooks that imbedded in the silk (Figure 22). When muscles M1 and M2 (Figure 24) retract the apex of the proleg during detachment (Grenier, 1949), central hooks move further from the silk than do hooks nearer the lateral plates (Figure 23). Bristles of lateral plates could then help to extricate these outside hooks from the silk because, even during full retraction of the apex, bristles point distally.

Detachment of anterior and posterior hooks of thoracic prolegs could require extra energy and effort, because bristles of lateral sclerites touch hooks in only about eighteen of forty total hook rows. Hora (1930, p. 212) noted a ". . . sudden sharp pull . . . ." when larvae detached their thoracic prolegs from silk, so apparently not all hooks are free after larvae retract their circlets.

Campaniform sensilla on thoracic prolegs probably detect stresses on the apex of the proleg, especially from muscles Mv2 (Figure 24), when these muscles pull on the circlet through the area of cuticle where these sensilla lie. During their looping locomotion, larvae keep themselves attached with posterior hooks of thoracic prolegs while they bring their anal prolegs from old to new attachment pads (Chapter 2). Sensory input from campaniform sensilla about tension in the cuticle between posterior hooks and insertions of Mv2 muscles (Figure 24) could assure larvae that their thoracic prolegs are securely attached. Since thoracic prolegs of black-fly larvae appear to have no other external sensilla, scolopophorous sensilla and other stretch receptors should be present in thoracic prolegs.

Figure 24. Diagram of muscles of thoracic proleg, left side, based on Grenier (1949) and Hinton (1955, 1963).

cs- campaniform sensillum,
i- muscular insertion,
Md, Mv2, M1, M2- muscles,
pl- lateral plate,
tc- thoracic circlet of hooks.



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This is the first sensillum reported from the thoracic proleg of any chironomoid larva. Campaniform sensilla could be useful for testing homology of thoracic prolegs within Culicomorpha, since Craig (1974, 1977) and Craig and Borkent (1980) found sensilla useful in determining homologies of structures of mouthparts among larvae in Culicomorpha.

5. Structure and function of the anal proleg

5.1 Introduction

When a black-fly larva filter-feeds, it is attached to the substrate by hooks of its anal proleg imbedded in a pad of silk the larva has applied to the substrate (Chapter 2). Larvae appear to spend little energy maintaining this attachment to pads, because when a molting larva pulls itself out of the previous larval skin, the exuviae stay attached (Cameron, 1922), and because dead larvae can be found still attached by their anal hooks (Wu, 1931). No one has yet explained why hooks of exuviae and dead larvae stay fastened to pads of silk. I propose that tubercles on anal hooks help to keep hooks imbedded in pads of silk.

Two disagreements about anal prolegs of black-fly larvae have not been resolved. Firstly, Puri (1925) and Grenier (1949) mentioned and Hora (1930) showed the presence of anterior and posterior gaps in anal circlets of hooks. Yet Dumbleton (1962) stated that anal circlets of hooks are continuous along the mid-ventral line. I will confirm the existence of both gaps.

The second disagreement concerns the number of apparent larval abdominal segments, and to which segment the anal proleg belongs. Puri (1925) counted eight, and Peterson (1978, p. 334) called the fused ventral papillae of *Stegopterna mutata* the "mid-ventral bulge of segment 8". On the other hand, Hinton (1955) stated that larvae had nine abdominal segments. Kölliker (1842) found ten abdominal segments in black-fly embryos; both Mecznikow (1866) and Craig (1969) found nine. Pupae have nine abdominal segments (Crosskey, 1969). Matsuda (1976) suggested that the ninth apparent abdominal segment represents the true tenth segment of larvae, pupae, and adults of nematoceran flies, the eighth apparent segment being made up of the eighth and ninth true segments. None of these authors showed precisely where this segment begins for black-fly larvae. I propose an intersegmental line for the beginning of the apparent ninth abdominal segment.

Anal prolegs of black-fly larvae of nearly all species bear sclerites proximal to the circlets of hooks (Grenier, 1949). Dumbleton (1962) showed some of the variation that can occur in these anal sclerites, and Crosby (1974) described and gave terms for details of their structure.

Grenier (1949) studied the anal proleg comprehensively and suggested that anal sclerites help larvae to pull their anal hooks out of silk. But the mechanism of detachment that he suggested would have hooks tearing through attachment pads of silk or pulling silk off of the substrate because anal hooks beside the anal sclerite point toward this sclerite. Such a detachment could require much energy, and I will show (Chapter 6) that vacated pads of silk are *not* torn. I will propose in this chapter a new mechanism and function for the anal sclerite.

5.2 Materials and methods

Materials and methods are those described in Chapter 3, with the following additions.

Abdomens of larvae killed in ethanol were soaked in alcoholic eosin, examined with a Wild M5 stereomicroscope, and sketched with the help of an eyepiece grid. Temporary slide mounts of anal sclerites and permanent mounts of abdomens were examined with a Leitz SM-LUX compound microscope and sketched with the aid of a camera lucida mounted on the microscope. Polarizing filters aided the search for muscles. Angles between muscles and between arms of anal sclerites were measured from the sketches.

A larva killed with boiling water while attached and feeding inside a beaker of stirred Sturgeon River water died still attached. The dead larva was then detached, prepared as previously described, and examined by scanning electron microscope.

5.2.1 Terms

Table A-9 lists terms for structures on and around the anal proleg.

5.3 Results

Posterior rows of hooks slant obliquely away from the posterior gap in the anal circlet of hooks (pg, Figure 26), and anterior rows in their turn slant toward the anterior gap (ag, Figure 27). The outer hooks in rows beside an anterior gap (ag, Figure 27) are closest to the gap, but the outer hooks in rows beside a posterior gap (pg, Figure 26) are farthest from the gap. That is, the first two or three rows of hooks to either side of a gap in the anal circlet of hooks have fewer hooks than do other rows (Figures 26 and 27).

The base (ba) of each hook is rounded and raised above its supporting cuticle (Figures 27 and 28). While the tine of an inner hook in a row of hooks is shorter than its base, the tine of an outer hook is longer than its base (Figure 27). Hooks central in a row bear up to seven tubercles (ht, Figure 28) while hooks at both ends of a row bear no tubercles (Figure 27).

The area of cuticle posterior to the anal organ (ao, Figure 25), and between the anterior arms of the anal sclerite (ar) is bare of bristles and tubercles (Figure 26), and convex (Figures 25 and 26). Scales of the anal sclerite (sc, Figure 26), visible with light microscopy, cover the cuticle beside the anterior arms. Up to six short anal sclerite sensilla (as, Figure 26) have their sockets on each side of the midline of the anal sclerite (ml), at the bottom of a V-shaped longitudinal depression in the sclerite (Figures 25 and 29a). Seven similar sensilla (as) lie in a row between each posterior arm and the adjacent hooks of the proleg. Laterodorsal expansions (lx) of the proleg hang over the posterior arms (Figure 25), and out beyond the tips

Figure 25. *Simulium vittatum* larva. Anal proleg, postero-lateral view, right side.
Scale=200 um.

Figure 26. Anal proleg, posterior view, anal organ retracted.
Scale=100 um.

Figure 27. Anal proleg, anterior midline.
Scale=20 um.

Figure 28. Hooks of anal proleg.
Scale=4 um.

af- anal flap,
ag- anterior gap in circlet of hooks,
ao- lobe of anal organ,
ar- tip of anterior arm of anal sclerite,
as- sensilla of anal sclerite,
ba- base of hook,
db- debris,
fc- flexible cuticle,
ho- hooks of proleg,
ht- tubercles on hooks,
lx- latero-dorsal expansion,
L1 through L3- furrows in abdominal cuticle,
ml- midline of anal sclerite,
pg- posterior gap in circlet of hooks,
pr- tip of posterior arm of anal sclerite,
sc- scales of anal sclerite,
si- silk,
vp- ventral papilla.

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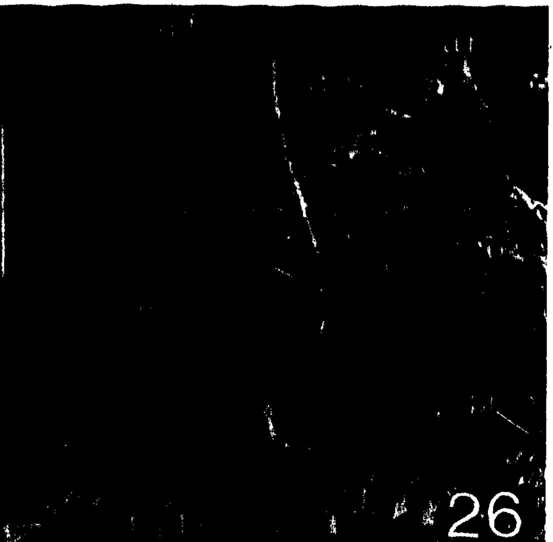

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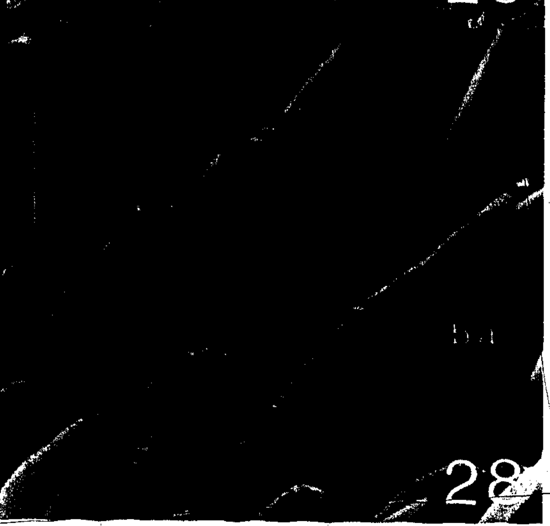

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of the posterior arms (Figure 29b). More than thirty setiform sensilla are spaced evenly over each laterodorsal expansion (Figure 26).

Three furrows (L1, L2, and L3; Figure 25) circle the abdomen anterior to the anal hooks. Along furrow L1, an area of flexible cuticle (fc) abuts less flexible cuticle of the eighth abdominal segment. Furrow L2 marks the anterior margins of the ventral papillae (vp), and the anal flap (af, Figure 25), which covers the anus and the anal organ. Furrow L3, posterior to the ventral papillae, extends almost to the tips of the anterior arms (ar) of the anal sclerite (Figure 25).

In dorsal view, anterior arms of anal sclerites are about 45° to posterior arms on the same side (Figure 29a). Muscles M2 (Figure 29b) run about 40° to each other; muscles M1 (Figure 29b) run about 15° to each other. Tips of posterior arms (pr) are near the eleventh row of hooks from the posterior gap (pg) in the circlet (Figure 29b). A pair of muscles that Grenier (1949) described as extending from the two large retractor muscles insert (i, Figure 29b) inside the circlet of hooks between the fifth and tenth hook rows on each side from the posterior gap in the circlet (pg). Grenier (1949) did not show their insertions with respect to the posterior gap.

On the larva killed while attached, the anterior part of the circlet was close to the ventral papillae so that much cuticle was in a deep fold between them, and the

Figure 29. Anal sclerite.

a) dorsal view,

b) posterior view.

Scale=100 um.

af- anal flap,

ar- tip of anterior arm of anal sclerite,

ho- hooks,

i- insertion of dorsal internal muscle of circlet of hooks,

lx- laterodorsal expansion of anal proleg,

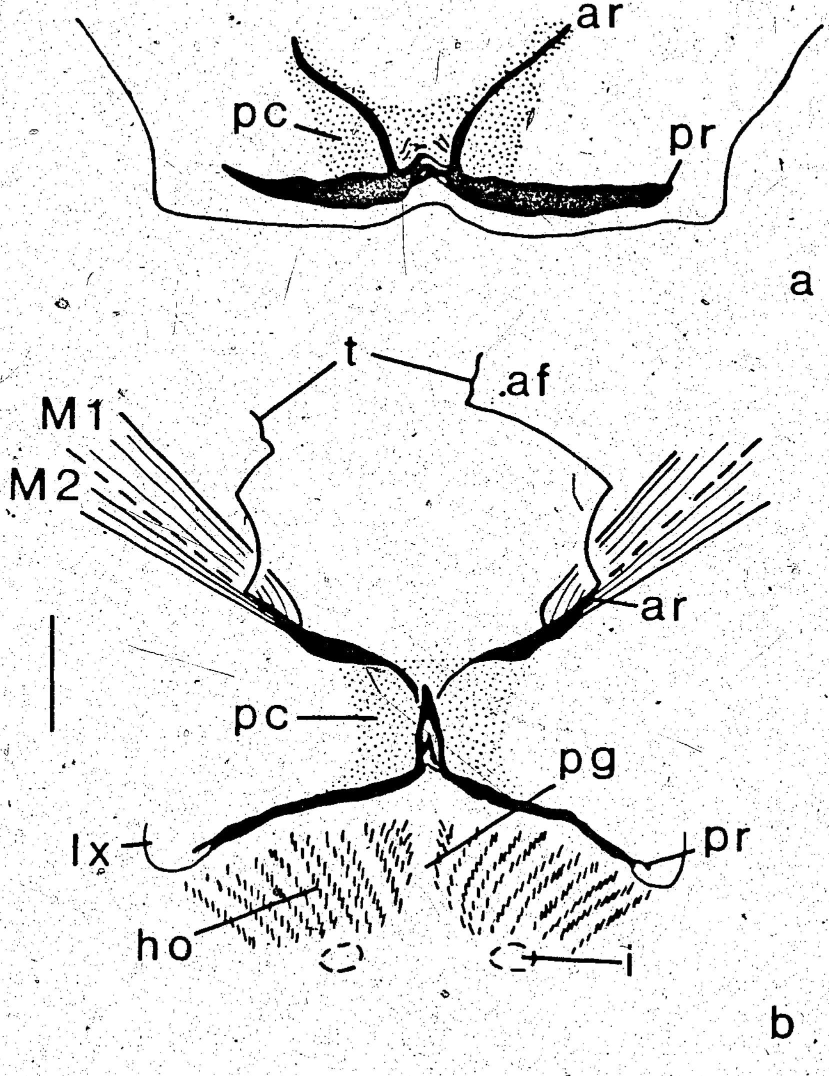
M1, M2- anal sclerite muscles,

pc- pigmented cuticle,

pg- posterior gap in circlet of hooks,

pr- tip of posterior arm of anal sclerite,

t- tear in anal flap to allow flattening of anal sclerite.



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flexible cuticle (fc, Figure 25) was folded up. The posterior arms of the anal sclerite were 180° to each other, so the attached circlet had a "D" shape in ventral view, the straight part of the "D" being the circlet along the posterior arms.

When the cuticular areas inside circlets of hooks of alcohol-preserved larvae were pushed anteriorly, the anal sclerites folded along their longitudinal midlines and midlines moved anterior to tips of the arms. On larvae that died with strongly retracted anal prolegs, the hooks at the posterior gap (pg) were pulled dorsal and anterior to the hooks at the tips of the posterior arms of the anal sclerite.

5.4 Discussion

5.4.1 Hooks of anal proleg

Puri (1925) imprecisely described hooks on thoracic and anal prolegs as arranged in "radial" rows. Tines of hooks point more or less radially, and since hooks point to one side of their rows, the rows are not radial but oblique. The arrangement of hooks to point to one side of a row allows many long hooks in a row to work together without interfering with each other. More hooks imbedded deep in the silk give larvae more reliable attachment.

At posterior gaps in circlets of hooks of thoracic prolegs and anterior gaps in circlets of hooks of anal prolegs, rows of hooks extend toward the respective gaps as the rows cross the circlet from inside to outside. These are usually the downstream gaps when their respective prolegs are attached (Chapter 2). Downstream hooks resist most of the horizontal force of the flowing water on larvae. This arrangement of rows could keep contra-lateral hooks at downstream gaps from interfering with each other.

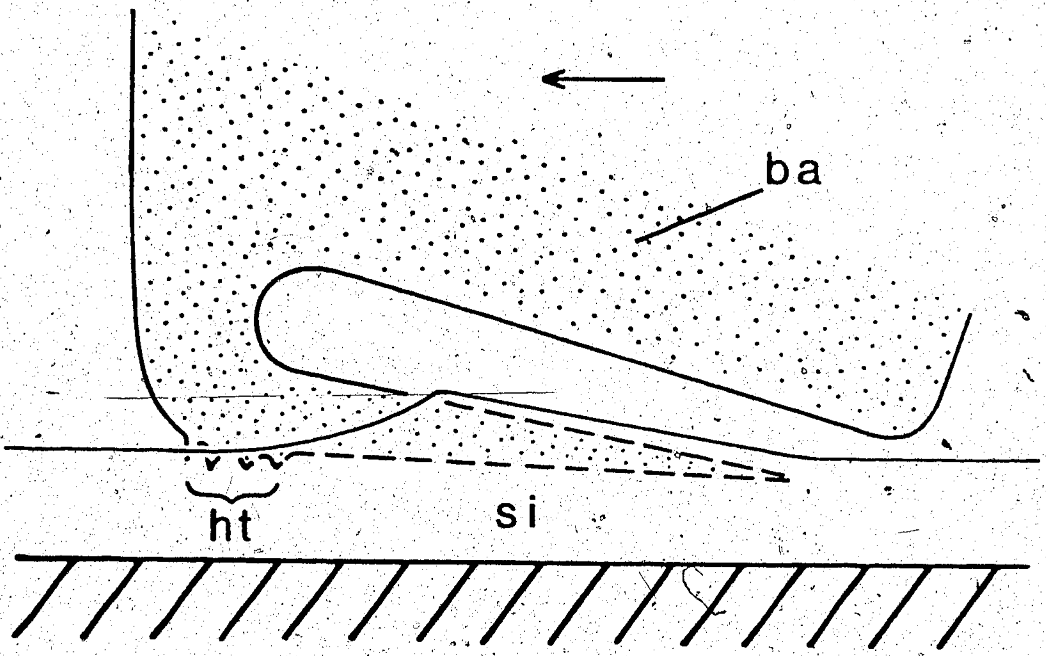
This study is the first to report tubercles on hooks of black-fly prolegs. These tubercles could have been overlooked before because it is difficult to isolate and mount a hook on its side for light microscopy and because hooks obscure each other. Tubercles on hooks probably imbed in pads of silk and help to keep these hooks from retracting once inserted (Figure 30). This helps to explain why exuviae (Cameron, 1922) and dead larvae (Wu, 1931) stay attached. With no pull at the centre of the anal proleg to disengage the tubercles from the pad of silk, the hooks probably cannot work loose. Larvae having hooks with tubercles would spend little energy maintaining their attachments. While thoracic hooks would save larvae energy by being easily retracted from sticky (Chance, 1970) and elastic fresh silk, anal hooks would save larvae energy by staying attached in older (less sticky and elastic) silk.

Figure 30. Diagram of attached hook of anal proleg, lateral view, with tubercles on hook imbedded in pad of silk. Arrow points to centre of circlet.

ba- base of hook,

ht- tubercles on hook,

si- silk.



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5.4.2 Scales of anal sclerite

Scales of the anal sclerite (sc, Figure 26) could help to clean the two lateral lobes of the anal organ, because lobes of the anal organ protract and retract over them. Scales of the anal sclerite in Figure 26 caught a filament of debris (db) stuck under the anal flap (af).

5.4.3 Sensilla of anal proleg

During attachment, mechanoreceptors on the laterodorsal expansions of the proleg probably respond to contact with the venter of the head capsule. Anal sclerite sensilla (as) beside the circlet could also tell larvae when the posterior midline of the circlet touches the substrate. Sensilla (as) along the midline of the anal sclerite are difficult to find by light microscopy, and as a result only Rubtzov (1959-1964) has illustrated them before. They probably detect particles caught in the groove in the midline of the anal sclerite. If anal sclerites hinge along this groove, it would be important for larvae to keep that groove clean.

5.4.4 Intersegmental line for ninth apparent abdominal segment

Furrow L1 is probably a functional annulation and not an intersegmental line. Firstly, it runs diagonally across the abdomen, whereas most intersegmental lines run at right angles to the longitudinal axis. Secondly, the flexible cuticle area (fc) folds as the anal proleg shifts ventrally when the larva attaches, and its suppleness permits great flexibility about the anal proleg (Chapter 2). This furrow probably marks where flexible cuticle meets more rigid cuticle. The flexible cuticle area posterior to L1, then, is probably part of the eighth apparent abdominal segment.

The ventral part of the anal proleg probably connects to the abdomen at furrow F3 (Figure 25). The tips of the anterior arms of the anal sclerite mark the dorsal border of the proleg. The dome of cuticle between the anterior arms of the anal sclerite (Figure 26) probably belongs to the anal proleg because part of it is the pigmented cuticle between the anterior arms of the anal sclerite (pc, Figure 29). Ross and Craig (1979, their Figure 17) show the anterior border of this dome of cuticle.

Furrow L2 (Figure 25) is probably the intersegmental line for the ninth apparent abdominal segment. It is probably an intersegmental line because it completely encircles the abdomen, because it has no obvious function as does furrow L1, and because muscles originate posterior to it (Puri, 1925). The segment behind furrow L2 is probably the ninth *apparent* abdominal segment because it probably contains remnants of the tenth true segment (Matsuda, 1976). Jobbins-Pomeroy (1916) and Smart and Clifford (1965) showed this line clearly. As well as the anus, this segment would also bear the ventral papillae, the anal proleg, and the anal organ.

5.4.5 Mechanism and function of anal sclerite

Grenier (1949) suggested that when muscles of the anal sclerite pull, hooks of the anal proleg nearest the dorsal midline detach first, then, with the sclerite working as a second class lever, hooks further and further along the posterior arms of the sclerite detach. As I interpret Grenier's (1949) mechanism, the pigmented cuticle lateral to the midline of the anal sclerite (pc) would have to flatten and stretch a great deal. Firstly, the anterior arms would move from 90° to each other (Figure 29b) to 40° to each other, so that the outside pair of anal sclerite muscles could pull at the anal sclerite midline as Grenier (1949) suggested. Secondly, the pigmented cuticle would have to stretch further to allow hooks near the posterior midline to detach before hooks further along the posterior arms detached.

Muscles inserting inside the circlet of hooks of the anal proleg should detach the hooks (Chapter 2), while muscles inserting outside the circlet should attach anal hooks because all hooks point radially, and because larvae probably pick up the heels of hooks to pull tubercles on hooks free of the silk (Figure 30) before trying to retract hooks. The anterior 2/3 of the hooks attach by contraction of muscles inserting outside the circlet. But no muscles insert at the outside edge of the posterior 1/3 of the circlet of hooks (Puri, 1925, his Figure 10). Anal sclerites could help larvae to attach their posterior hooks in the following way.

If during detachment the dorsal internal circlet muscles (i, Figure 29b) contracted, they would pull the anal sclerite midline anteriorly as well as dorsally. This would disengage the hooks near the posterior midline (Figure 31a). The sclerite would close around the anal sclerite sensilla (as) along the midline (ml, Figure 26), and the domed cuticle between anterior arms (Figures 25 and 26) would flatten as the arms swung apart.

Similarly, if the dorsal internal circlet muscles (i) relaxed, and anal sclerite muscles (am, Figure 29b) pulled on the anterior arms, haemolymph pressure could push the anal sclerite midline posteriorly (Figure 31b). The anal sclerite posterior arms would thus pull hooks near their tips laterally and hooks near the posterior midline posteriorly (Figure 31b), and into the attachment pad (si, Figure 26). For this to work effectively, anterior hooks of the anal proleg would have to be hooked in the pad of silk before the anal sclerite begins to straighten.

According to this new mechanism, the anal sclerite midline would flex away from the substrate as it flexed anteriorly, because abdomens of detaching larvae are angled from the substrate. The midline of the anal sclerite would be the first to leave the substrate on detachment. Such an observation could have inspired Grenier to propose his (1949) mechanism for the function of the anal sclerite.

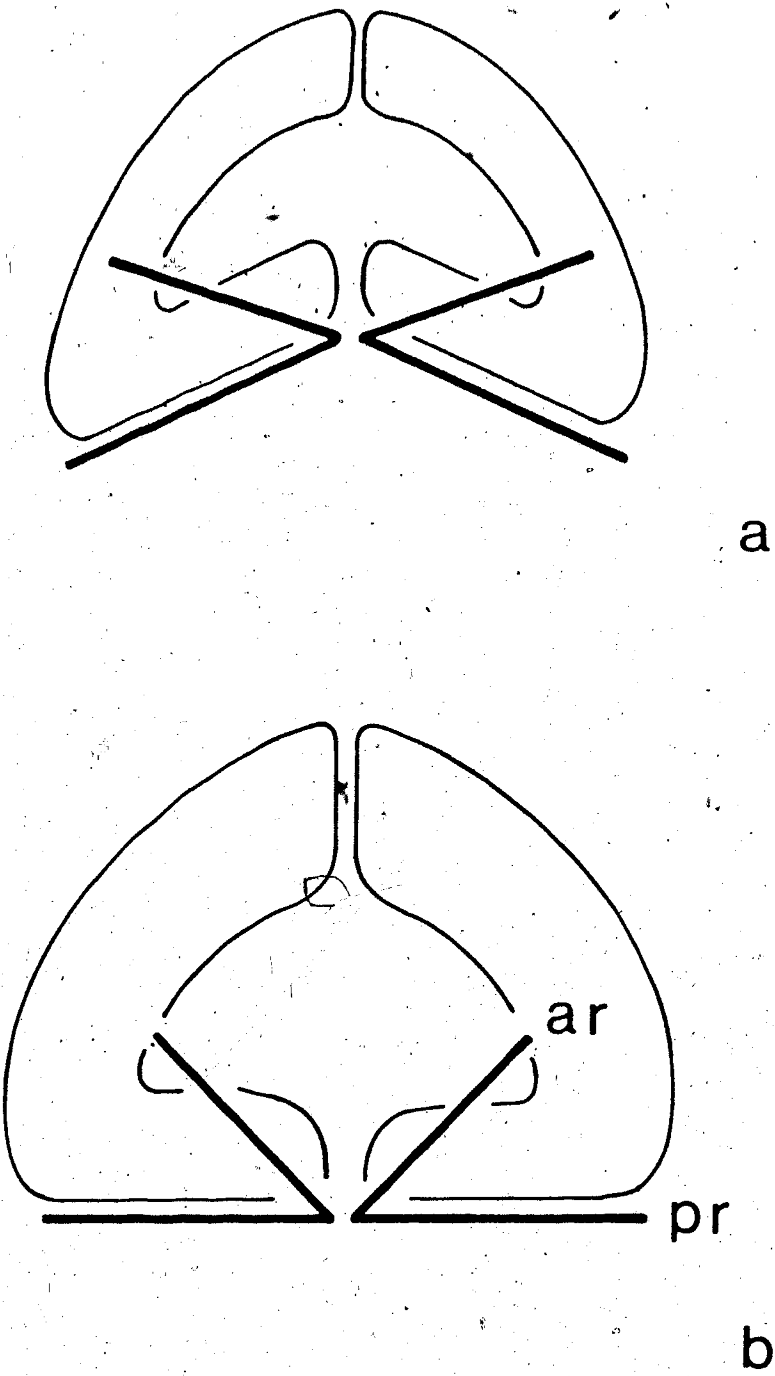
Figure 31. Proposed function of anal sclerite of simuliid larvae; attachment of posterior hooks of anal prolegs, dorsal view.

a) midline of sclerite flexed anteriorly, posterior hooks not attached.

b) midline unflexed, posterior hooks attached.

ar- tip of anterior arm of anal sclerite,

pr- tip of posterior arm of anal sclerite.



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Posterior hooks of the anal proleg must resist forces pulling dorsally. The straight shape of the posterior part of the attached anal circlet of hooks (Figures 3 and 31b) should allow larvae to distribute these forces more evenly among posterior hooks than if these hooks attached in an arc.

Larvae with anal sclerites lacking posterior arms (*Gymnopais* and *Twinnia*; Wood, 1978) would attach their posterolateral hooks less strongly, but presumably would have greater flexibility about their anal prolegs. *Ectemnia* larvae lack anal sclerites, but their large abdomens (Peterson, 1978) would rest on the substrate, aiding their attachment. First-instar larvae also appear to lack anal sclerites (Craig, 1975; Crosby, 1974; Davies, 1965; Dumbleton, 1964), but detaching forces could be weak on such small larvae, as they are well within the boundary layer.

6. Pads of silk

6.1 Introduction

Early students of black-fly larvae (Planchon, 1844; Verdat, 1823) believed that larvae attached directly to the substrate by suction of the anal proleg. Newstead *et al.* (1907) first noted that locomoting larvae attach the anal proleg to silk spun on the substrate, but until Tonnoir (1923) described this in detail, others still assumed (*e. g.*, Jobbins-Pomeroy, 1916) that the anal proleg was a sucker. The opinions, that suction aided attachment, with silk sealing spaces between hooks of the anal proleg (Puri, 1925), or with abdominal folds sealing to the substrate around pads (van Oye, 1936), persisted but gave way before the observations of Hora (1927a; 1930) and later Grenier (1949) on larval behaviour and structure.

Pads of silk applied to the substrate have received little attention. Although Hora (1927a) illustrated pads of silk, larvae made them in still water, and attached only their mouthparts and thoracic prolegs to them (Hora, 1927b). Lewis (1960) sketched a pad of silk produced, in flowing water, under laboratory conditions, by a *Simulium neavei* Roubaud larva, but he distinguished only the rim and rows of marks where hooks of the anal proleg had attached.

In this chapter, I will describe in detail pads spun by larvae in flowing water in the laboratory and in a river.

Laboratory observations will allow me to explain features of pads in terms of larval spinning behaviour. I will show that larvae conserve their silk. Field results will allow me to infer how often larvae were obliged to spin new pads as old pads began to rot.

6.2 Materials, methods, and study site

6.2.1 Sampling pads of silk spun in the laboratory

Simulium vittatum larvae collected at the field site (Chapter 3; Table A-10) were kept in rearing tanks at 1-4°C for two weeks, then transferred to a cylindrical glass jar (10 cm x 8 cm high). The jar was half filled with water from the field site, filtered through a wad of cotton. Microscope slides standing on end lined the jar. Air bubbled from the bottom of the jar produced a weak current that encouraged larvae to attach to the slides. The bubbling was stopped before a stirring bar in the jar was started spinning by a Fisher Flexa-mix magnetic stirrer. The resulting current in the jar, measured by a Kent mini flow meter, was 4-7 cm/sec, and larvae filter-fed inside.

Black-fly eggs were collected 26 Sept. 1979 at the field site from leaves of *Typha* sp. After storage at 2-7°C for a day, then in bubbled water at room temperature for a day, then in standing water at room temperature for a day, eggs hatched. The eggs were probably *S. vittatum* eggs because hatching after a set of such weak stimuli so late in the year (ice begins to cover the Sturgeon River in late October, Appendix Table A-11) implies a species of black-fly that overwinters in the larval stage, and *S. vittatum* is the only species in the area that does so (Abdelnur, 1968). The resulting first-instar larvae were introduced to the jar as previously described, except that coverslips had been glued to some of the slides with silicon sealant. The coverslips were later broken and fit into the scanning electron microscope. Behaviour of larvae inside the jar was observed and noted.

6.2.2 Sampling pads of silk spun in the field

The field site was in central Alberta, Canada, 25 km NW of Edmonton (Appendix Table A-10). The Sturgeon River there was a small, warm, slow river (Appendix Table A-11), with fairly clear, well-oxygenated water (Appendix Table A-12), supporting many filter-feeding animals (Appendix Table A-13).

Stakes 3 x 2 x 120 cm were driven into the substrate 1 m apart upstream and downstream and 30 cm apart to the sides. A clothespin was lashed to each stake so that it opened upstream, a few centimeters below the surface of the water, or at the stream bottom, or halfway between. A pair of microscope slides glued back to back with silicon sealant was held in each clothespin at the downstream lower quarter of each pair. Pairs of slides could later be separated to allow examination of pads on both slides of the pair. Plant debris caught on the slides and stakes was removed twice daily. Nine pairs of slides were exposed to the river for each of 1/2, 1, 2, and 5-day periods. Some of the slides on the bottom were accidentally crushed, others were dislodged and lost. Strips of clear polyethylene (1 m x 2.5 cm x 0.4 mm) were attached at one end to ceramic tiles laid on the stream bottom. Water velocity at the trailing end of the strip was 35-45 cm/sec (Table A-11). Water velocity among the stakes was 20-30 cm/sec. To obtain samples of pads on a natural substrate, leaves of *Potamogeton richardsonii* with larvae attached were collected.

6.2.3 Storing and examining pads of silk

Larvae were washed from slides under a stream of distilled water and the slides stored in plastic slide racks immersed in 98% ethanol. Seven cm of polyethylene tape was cut from the end of the strip, and stored with larvae still attached in 98% ethanol. Leaves of *P. richardsonii* were also rolled up with the larvae still attached and stored in a vial of 98% ethanol. Most larvae detached on immersion in 98% ethanol. Pads of silk on slides, polyethylene, and leaves were stained for five minutes in a saturated solution of Buffalo Black (Naphthol Blue Black) in 98% ethanol, rinsed in fresh 98% ethanol, placed in a Petri dish again with 98% ethanol, and examined with a Wild M5 stereoscopic microscope. Pads so treated looked blue.

Decomposition of pads of silk was scored in the following way. Pads were not considered to have begun rotting until the rim (ri, Figure 34) of the pad had been affected. Bits of silk recognizable as pads, but more than half rotted, were so recorded. Proportions of unrotted (Figure 34), less-than-half-rotted (Figure 37), and more-than-half-rotted pads on each pair of slides, were determined. Arc-sine square root transformations [illegible] proportions were applied (Sokal and Rohlf, 1969) and means and intervals of standard error were calculated according to period of exposure to the river. These statistics were then transformed back to proportional values and plotted.

Pairs of slides were separated and pads attached were mounted in Canada Balsam. Photomicrographs were taken on Kodak Plus-X Pan 135 film with a Leitz Wetzlar Orthomat camera on a Leitz Wetzlar Ortholux compound microscope. Pads spun by first-instar larvae were photographed with phase-contrast microscopy.

Pads were measured at their greatest width across the rim (ri) parallel to the flat side of the D-shaped area (Da, Figure 34).

6.2.4 Measuring thickness of pads and scanning electron microscopy

A minuten pin dragged across a coverslip was used to tear pads of silk first-instar larvae had spun. This was also done to especially darkly-stained attachment pad of last-instar size on the polyethylene strip. Both samples were critical-point dried directly from 98% ethanol, sputter-coated with gold, and examined with a Cambridge S150 scanning electron microscope at 16-20kv. Photomicrographs were taken at low angles of view with Kodak Plus-X 120 roll film.

6.3 Results

6.3.1 Features of pads

Pads of silk of *S. vittatum* larvae (Figures 32 to 38) have three main features: a c-shaped area (ca), a D-shaped area (Da), and a peaked area (pa). Only a trace of silk connects the c-shaped area, downstream on most pads, to the larger D-shaped area (Da) that is bounded on many pads by a rim (ri). The diatom (di) in Figure 38 rests on the rim (ri). On pads from first-instar larvae, the D-shaped area was more oval than D-shaped (Figure 36).

On examination by scanning electron microscope, pads appear to be single masses of silk, with no sign of filaments, threads, or weaving (Figure 38). On the periphery of the D-shaped area, inside the rim, are slanting rows of holes (lh, Figure 38) in the silk. I found holes of such shape on no other parts of pads. At the mouths of some of these holes can be found a row of smaller holes (sh) in the silk (Figure 38). Outside the rim, pads thin quickly,

Figure 32. *Simulium vittatum* larva. Feeding pad, spun under laboratory conditions.
Arrow points downstream.
Scale=100 um.

Figure 33. Locomotory pad, spun under laboratory conditions.
Arrow points downstream.
Scale=100 um.

Figure 34. Pad spun on a glass slide suspended in the Sturgeon River.
Arrow points downstream.
Scale=100 um.

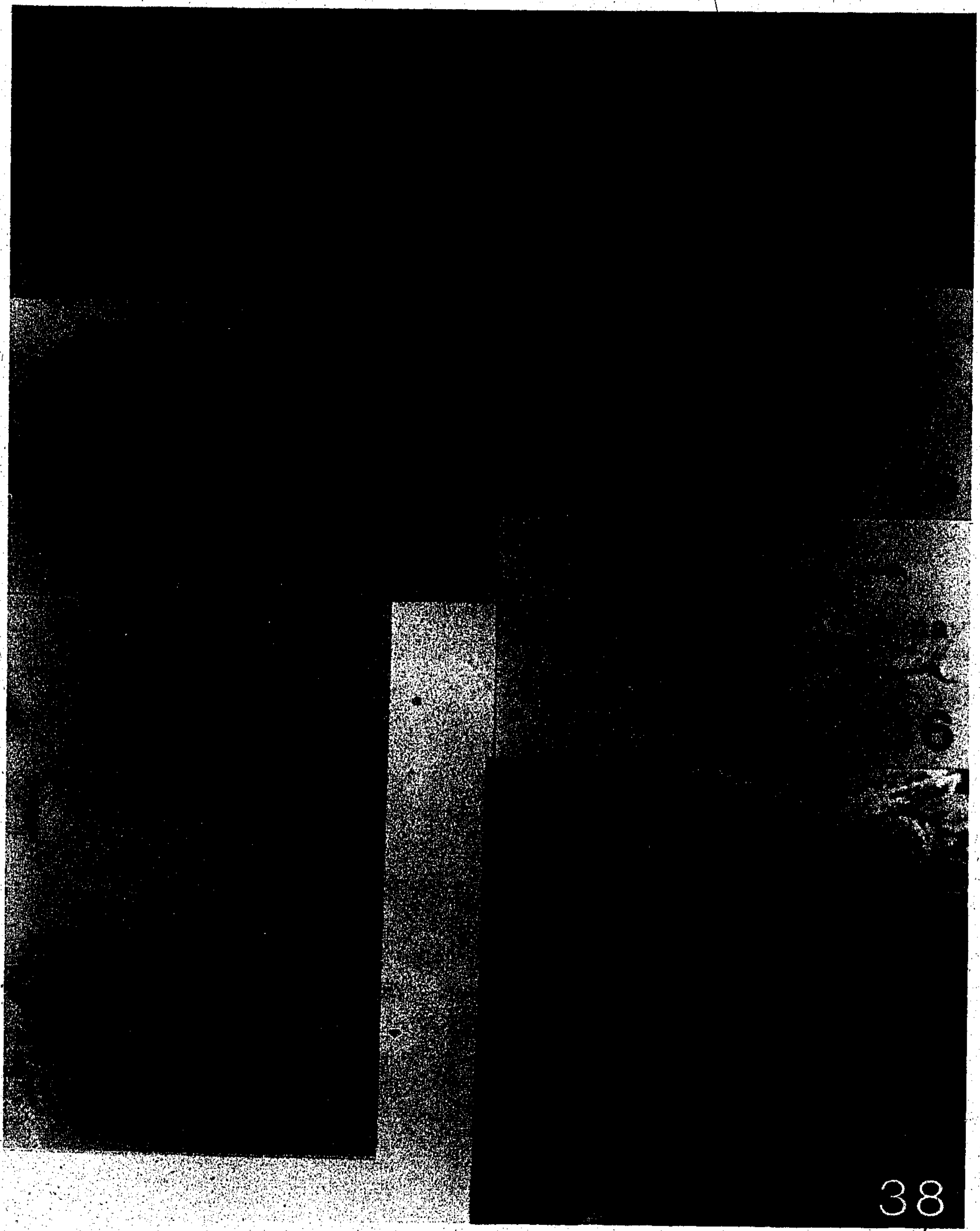
Figure 35. Pad spun on a glass slide suspended in the Sturgeon River.
Arrow points downstream.
Scale=100 um.

Figure 36. *Simulium* sp., probably *S. vittatum*. Pad of a first-instar larva, spun under laboratory conditions.
Arrow points downstream.
Scale=20 um.

Figure 37. Two rotting pads from the Sturgeon River, probably spun by the same sixth-instar larva, rotting.
Arrow points downstream.
Scale=100 um.

Figure 38. Detail of pad from the Sturgeon River.
Scale=5 um.

ca- c-shaped area of pad of silk,
Da- D-shaped area of pad,
di- diatom,
lh- larger hole in pad,
pa- peaked area,
rg- ridge leading to peaked area,
ri- rim,
ro- area of pad rotted away,
sh- smaller hole in pad.



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except downstream of the D-shaped area on some pads (*e. g.*, Figure 37, upper pad), and upstream of the the D-shaped area of all pads.

Midway along the upsteam parts of D-shaped areas is a pair of connected peaks (pa, Figures 32 to 37) of silk. On many pads, peaks had fallen to one side or the other (*e. g.*, Figure 35). Leading away from each peak, laterally and upstream, is a ridge (rg, Figures 32 to 36) of silk.

Pads spun by last-instar larvae were about 400 um in diameter, and about 100 um across the empty part of the c-shaped area (Figure 32). The pad on the strip of polyethylene from the Sturgeon River was about 3 um thick just inside the rim and about 10 um thick through the rim, and less than 1 um thick outside the rim. Pads spun by first-instar larvae were about 40 um across (Figure 36), and the one measured was about 0.3 um thick just inside the rim.

The pad in Figure 35 is made of pairs of daubings of silk, less evident in Figure 33, but quite visible inside of and upstream of the rim (ri) in Figure 32. At no point on attachment pads did I recognize torn silk (Figures 32 to 37).

6.3.2 Pads of silk spun in the laboratory

The same larva spun the pads of silk in Figures 32 and 33. The pad in Figure 32 was spun while the larva was attached to the pad in Figure 33. The larva filter-fed while attached to the pad in Figure 32, but did not filter-feed while attached to the pad in Figure 33. Pads larvae were attached to while filter-feeding were shaped like that in Figure 32, and pads that larvae were attached to while they moved about were shaped like that in Figure 33. Pads of silk larvae attached to while filter-feeding I call "feeding pads," and pads larvae attached to while shifting positions I call "locomotory pads." Feeding pads stained more darkly, and therefore contained more silk, than locomotory pads. No locomotory pad had a rim around the D-shaped area (Da, Figure 33). Locomotory pads were less strongly oriented to the current (*e. g.*, Figure 33, note arrow). A trace of silk linked many consecutive locomotory pads. Only when larvae were detached by force were the pads torn.

6.3.3 Pads of silk spun in the Sturgeon River

Most pads of silk sampled from the Sturgeon River (*e. g.*, Figure 34) were similar to that in Figure 32. Others (*e. g.*, Figure 35) were similar to the pad in Figure 33. Pads found on rocks and on leaves of aquatic plants in the Sturgeon River were similar to those found on the slides, except that all had rims. Pads spun in the Sturgeon River stained more darkly, and those with rims (ri) had more complete rims than those spun in the laboratory (compare Figure 34 with Figure 32, and Figure 35 with Figure 33). Pairs and trios of pads with rims could be recognized on slides exposed to the Sturgeon River. These pads were close to each other, the same size, (the closest pads of similar size were usually some distance away), and their rims had similar shapes. Figure 37 shows such a pair.

Pads in the Sturgeon River rotted. One of the first signs of rot was loss of the c-shaped area (Figure 37). Rarely, small areas all over pads began to rot at about the same time (ro, Figure 37, darker pad). Commonly, pads rotted inward from points along the edge (ro, Figure 37, both pads), the silk obliterated along rounded fronts.

About 20% of pads ≤ 1/2 day old showed significant signs of rot (Figure 39). About 40% of pads ≤ one day old showed signs of rot and two pads were more than half-obliterated (Figure 39). Of pads ≤ two days old, about 20% were more than half rotted away (Figure 39). The proportions of pads without rot dropped steadily until the second day (Figure 39).

6.4 Discussion

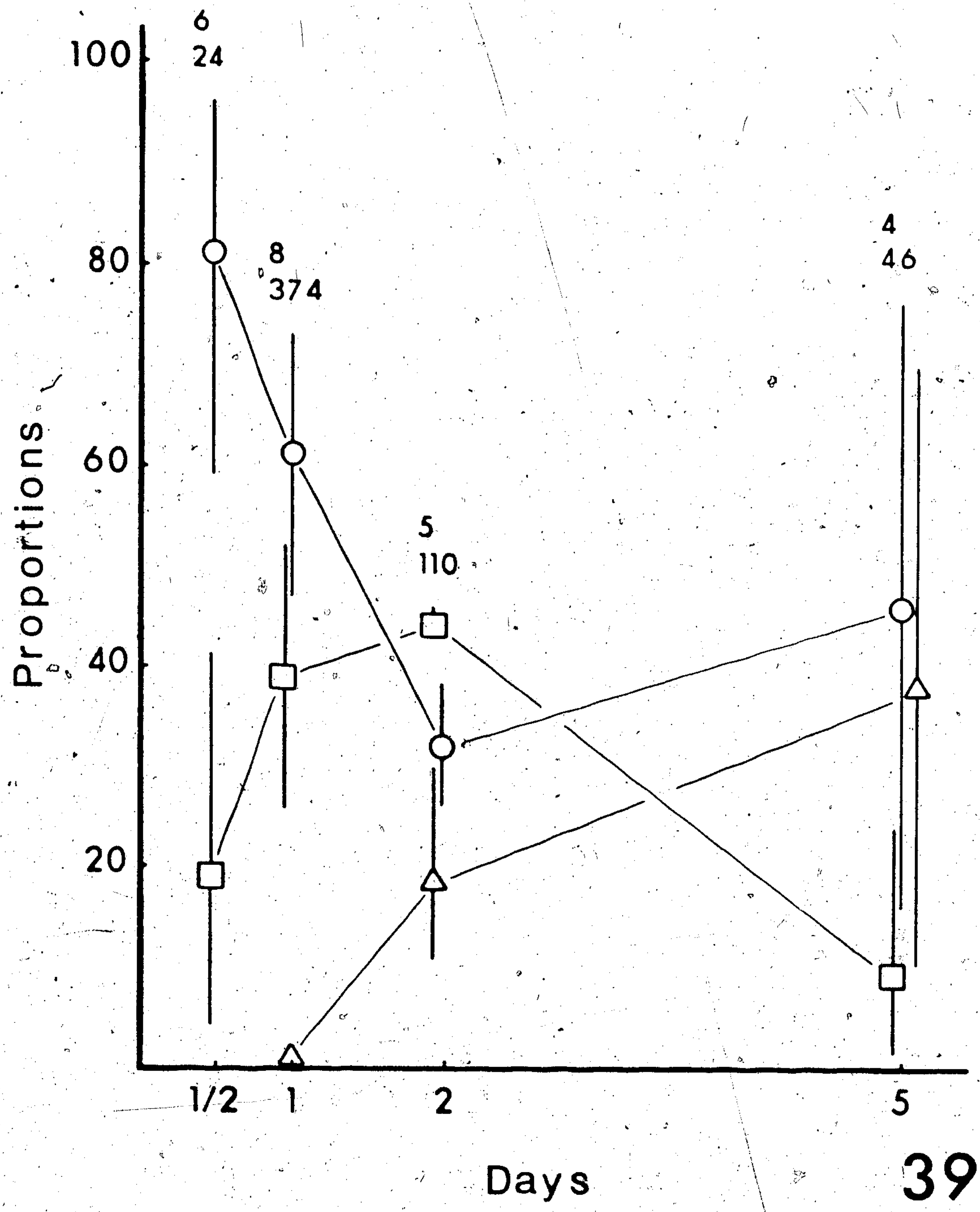
6.4.1 Interpreting features of pads of silk

Comparing features of pads with sizes and shapes of mouthparts and prolegs, and referring to what is known of larval locomotory behaviour, one can attempt to interpret the features of pads of silk.

Larvae begin to spin pads of silk by attaching the thoracic proleg to silk applied to the substrate and then

Figure 39. Proportions, expressed as percentages (mean ± standard error), of rotting pads of silk of *Simulium vittatum* larvae on microscope slides suspended in the Sturgeon River for up to five days.

circles- pads with no rot affecting their rims,
squares- pads rotting, but less than half-rotted,
triangles- pads more than half-rotted.
Top numerals above error bars=number of slides recovered.
Lower numerals above error bars=total number of pads counted.



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applying more silk just upstream of that attachment (Chapter 2). Because of its downstream position and size, the c-shaped part (ca) of pads (Figures 32 to 36) corresponds to the attachment site of the thoracic proleg. The indentation of the c-shaped area spun by a last-instar larva is about 100 um wide, nearly as wide as the tip of the thoracic proleg of a last-instar larva, about 110 um wide (Chapter 5). The c-shaped area had no marks to show where hooks attached, perhaps because thoracic hooks detach while the silk is still fresh (Chapter 2). The thoracic proleg probably attaches to silk at the c-shaped raised edge (Figures 32 to 36).

In my study of looping (Chapter 2), I could not be sure whether the thoracic proleg pulled silk from the mouthparts and applied it to the substrate for its own attachment, or if the mouthparts applied silk to the substrate, and the thoracic proleg attached later. If the thoracic proleg pulled silk from mouthparts and applied it to the substrate, the silk left behind would be in a circular or rectangular pile. Instead, this silk is in a semi-circle. This suggests that larvae do so directly from their mouthparts, corroborating this aspect of the observations of Puri (1925), Rubtzov (1962), and Smart (1934).

By shape, orientation, size, and structural detail, the D-shaped area corresponds to where the anal proleg attaches to pads. Circlets of hooks of anal prolegs, when attached, are also D-shaped (Chapter 2 and Chapter 5). The straight part of the "D" is upstream of the curved part on feeding pads and on the anal proleg of filter-feeding larvae (Chapter 2). A pad spun by a last-instar *S. vittatum* larva is about 400 um in diameter, as is the circlet of hooks of the anal proleg of a last-instar *S. vittatum* larva (Chapter 5).

Rows of holes (lh, Figure 38) in pads correspond in position and arrangement to rows of hooks of anal prolegs. I suggested (Chapter 5) that tubercles on hooks of anal prolegs imbed in pads of silk, preventing those hooks from retracting. The smaller holes (sh, Figure 38) at the mouths of larger holes (lh) in the pad are likely where tubercles of hooks penetrated the pad (Figure 30).

The anal proleg expands as it attaches to a pad (Chapter 5). As it expands, it could push silk, probably still fluid, to the outside of the pad, and so form the rim. Locomotory pads lack rims probably because they are spun of so little silk.

By their position and structure, the connected peaks (pa, Figures 32 to 37) on pads are where mouthparts attached. When the anal proleg attaches to a pad, it slides in behind the submentum and mentum (Chapter 2). Thus attachment of mouthparts on pads should be upstream and close to the D-shaped area. On part of the rim around the D-shaped area, the peaks are certainly close to where the anal proleg attaches. Larvae almost certainly plant their mental teeth in the pad, but the peaks, about 60 um apart on pads spun by last-instar larvae (Figures 32 to 35), are closer together than the corner teeth of the mentum, about 100 um apart.

Since mandibles meet the mentum near the midline, apices of adducting mandibles could gather silk on their aboral sides. Thus the silk making up peaks seems to be gathered from that spun just upstream of the D-shaped area. Oblique thickenings of this silk leading downstream and medially to the peaks suggest the oblique paths of mandibular teeth swinging toward the mentum. If this is true, the similarity of shape of the peaks contradicts my earlier observation (Chapter 3) that a pair of mandibles, when adducted at the same time, cross before reaching the mentum. Mandibles probably keep holding onto the pad until the anal proleg has attached.

Some features of the pads give clues as to how the silk was spun. Pads are not woven meshes of filaments of silk as Hinton (1955), Hynes (1970) and Smart (1934) suggested, but are solid (Figure 38) masses of silk. Viewed with light microscopes, pads appear woven of many fine filaments of silk (Figures 32 to 37), but the variations in thickness producing this effect are probably the result of larvae stretching silk between the daubings and blobs they apply to the substrate. On the other hand, pads of *Simulium loutetense* Grenier and Ovazza are woven networks (Lewis, 1960). Those larvae live in especially strong current (Lewis, 1956), so the weave could help the pad absorb shocks to larvae. Turbulent currents buffet larvae (Craig and Chance, 1982), and pads help to absorb the shocks (Chapter 2).

Paired daubings that seem to make up some pads (Figures 33 and 35) suggest that maxillae, implicated in helping to draw silk out of the salivary meatus (Chapter 3), apply silk to the substrate.

6.4.2 Feeding and locomotory pads of silk

I conclude from their similarity to feeding pads spun in the laboratory (Figure 32), that pads spun in the Sturgeon River that have rims (Figures 34, 37, and 38) are also feeding pads. Pads without rims spun in the Sturgeon River (Figure 35) were similar to locomotory pads spun in the laboratory (Figure 33). This suggests that pads without rims spun in the Sturgeon River are locomotory pads.

Assuming that all larvae from this population produce the same silk, pads staining more darkly are thicker and made of more silk. Although Craig and Chance (1982) implied that larval streamlining and larval behaviour during feeding reduced drag, the greater thickness (darker staining) of feeding pads suggests that larvae experience more drag when filter-feeding than they do when moving about.

Larvae conserve energy by expending less silk on locomotory pads than on feeding pads. Outside the rim a pad is a third as thick as it is inside the rim. Comparing the staining inside and outside the rim of the pad in Figure 34 with the staining of the pad in Figure 35, I estimate that locomotory pads contain a third the amount of silk that feeding pads contain. If locomotory pads must resist weaker forces for shorter periods than feeding pads must, larvae can, within limits, spend less silk on locomotory pads without increasing risk of detachment. The difference in thickness between laboratory-spun and river-spun feeding pads was perhaps due to the stronger (about five times faster) current in the river. If this were so, larvae could conserve silk by spinning out less silk for pads when feeding in weaker currents. Larvae also save silk by applying it only to discrete areas on the substrate, where the prolegs and mouthparts attach.

Silk, a secretion, is a form of "elimination", and a drain on an arthropod's pool of net production (Batzli, 1974). Larvae conserving silk can save energy for growth and reproduction. Larvae that conserve their reservoirs of silk also increase their range of movement. Conserving net production and maximizing range of movement could both contribute to selection for larvae that spin thin pads of silk.

6.4.3 Rate of rot of pads of silk

Rather than scraping pads up and eating them, as they do mats of algae (Chance, 1970; Kurtak, 1978; Mokry, 1975; Peterson, 1956; Serra-Tósio, 1967), black-fly larvae leave pads behind to rot. If larvae browsed on abandoned pads, one would expect pads to suffer straight tears where mandibles scraped at them. I found only ragged damage characteristic of rot. No larva I observed touched its recently-abandoned pads, let alone scraped at them. Despite these observations, larvae could still recycle nutrients and energy in silk in this way.

Perhaps a special conformation of molecules in silk resists digestion by most bacteria (and by black-fly larvae); perhaps the small volume of nutrient in a pad is not worth recycling. Certainly, newly-spun locomotory pads would be stuck strongly to the substrate, and contain little silk.

A larva that is feeding well at its site, and is not disturbed, probably stays attached to its pad until the the pad rots too far for the larva to depend on it. Then the larva probably spins a pad close by and attaches to it. This behaviour would produce the observed pairs and trios of pads in different stages of rot. Once rot progresses beyond the rim, hooks at that spot no longer engage silk; the attachment has become less secure.

Variability in rates of rotting, varying rates of net colonization of slides, and differing rates of spinning pads among larvae all contribute to uncertainty about average proportions of rot in Figure 39. Drifting vegetation fouling slides probably disrupted colonization. Also the slides were too small to gather many pads. Some slides had single pads on them. On such a slide, if the pad there was half-rotted, for example, the proportion of half-rotted pads on that slide would be 100%. Slides with only one or two pads thus affected the mean values more than did slides with many pads. Because some pads more than half rotted are beyond recognition as pads, numbers of pads more than half rotted would be underestimated, especially on slides exposed for five days.

Despite their variability, the proportions in Figure 39 give a picture of the onset of rot on an artificial substrate in the Sturgeon River, assuming that the pads most rotted on the slides were the oldest there.

About 20% of the pads at most 1/2 day old, and about 40% of the pads at most one day old, had begun to rot appreciably. Some pads at most one day old were more than half-rotted. Assuming that larvae risk detachment if they filter-feed from pads whose rims had begun to rot, larvae probably replace their pads every half to full day. Pads could disappear in as little as two days, and would certainly disappear in three.

If decomposers colonize from nearby on the substrate, rot of pads on natural substrates would begin more quickly than on artificial substrates. By scraping the substrate of particles before laying down pads (Chance, 1970), larvae could be slowing the onset of this rot. On the other hand, if decomposers colonize pads from the cuticle of larval prolegs, onset of rot on artificial and natural substrates would be similar. Bacteria similar to those living on the exudate of a sensillum at the base of antennae of larvae (Craig and Batz, 1982) could cause rot, but whether or not decomposers are even bacteria is unknown.

Wu (1931) found that *S. vittatum* larvae in her stream, somewhat warmer (18-26°C) than the Sturgeon River (17-22°C), passed through the larval stage in thirteen to seventeen days. If feeding pads of last-instar *S. vittatum* larvae are 3 um thick, and last-instar larvae average 6766 um long (Ross, 1979), a feeding pad is a loss of less than 1/2000 of a larva's total volume. If feeding pads of first-instar *S. vittatum* larvae are 0.3 um thick, and first-instar larvae average 651 um long (Ross, 1979), this ratio seems to stay constant throughout the larval stage. Assuming that pads rot at the same rate no matter their thickness, and that a larva must spin one or two new feeding pads every day, I suggest that black-flies probably spend less than 1% of net production through the whole larval stage to replace rotting pads, much less than the 14% of net production aphids spend on exuviae (Llewellyn, 1972).

6.4.4 Further work

Pads can now be sampled, preserved, and interpreted as records of larval locomotory behaviour. A larva's progress and bouts of feeding can be determined from the locomotory and feeding pads it leaves behind. Provided a larva does not detach, pads can show where, how, and how much that larva moves in a day.

The record of silk attached to the substrate is not yet completely interpretable because at least four structures of silk await discovery and study. When alarmed, larvae bend sideways and attach their mouthparts and thoracic proleg to the substrate to one side of the attached anal proleg (Chance, 1970; Grenier, 1949), they must leave some silk behind. Another structure of silk is the point of attachment of the anchored line (Chapter 3), and a third is the part of an anchored line that a larva has braked on (Chapter 3). A fourth structure of silk is the record of how larvae reattach to the substrate after drifting.

7. Larval silk and cocoon silk are different substances

Many insects spin cocoons in which they pass the pupal stage. Last instar larvae (or pharate pupae) secrete and store silk for making these cocoons in their labial glands. Before secreting cocoon silk, labial glands in larvae secrete, depending on the species, saliva, or larval silk. Black-fly larvae spin silk to attach themselves to the substrate (Hora, 1927a; Lewis, 1956; Puri, 1925; Smart, 1934; Tarshis and Neil, 1970; Tonnoir, 1923). Black-fly pharate pupae (Hinton, 1958) spin silk for cocoons (Burton, 1966; Peterson, 1956).

Even though MacGregor and Mackie (1967) described only one type of secretory cell in labial glands of black-fly larvae, they hinted that cocoon silk and larval silk could differ, and Chance (1970) suggested, on visual evidence, that cocoon silk is of different composition from larval silk. This chapter presents further evidence of this difference.

7.1 Materials and Methods

7.1.1 Silk Development and composition

Simulium vittatum larvae collected in the Sturgeon River, 25 km NW of Edmonton, Alberta, Canada (Appendix Table A-10) 23 August 1979 were preserved in vials of 98% ethanol. Larvae with abnormal fat bodies, or with nematodes or external growths were removed from the sample.

7.1.1.1 Development

Larval instar was determined from post-genal length (Ross, 1979). Thorax size of last instar larvae, normal, or large (due to growing imaginal discs), was also noted. Labial glands were removed intact from the larvae, epithelial sheaths (gl, Figure 40b) removed from around the contents, now solid, and colour and transparency of the contents were compared. Contents of some glands were mounted unstained in Canada balsam and photomicrographed on Kodak Plus-X Pan 135 film with a Leitz Wetzlar Orthomat camera on a Leitz Wetzlar Ortholux compound microscope.

7.1.1.2 Amino acid composition

Preserved contents of glands were pooled in four samples; (1) silk from fo [illegible] h instar larvae, (2) brown silk from seventh (last) instar larvae, (3) clear and opaque (mostly opaque) silk from seventh instar larvae, and (4) clear and opaque (mostly clear) silk from seventh instar larvae. Clear silk surrounded regions of opaque silk

(Figure 40c), so the two were difficult to separate. Each sample was sonicated to remove bits of epithelium clinging to the contents, then air dried. Samples were digested in 6 N HCl with 0.1% phenol at 110° C and in a vacuum oven for 24 hours. Except for sample (4), where only a concentrated sample was run, two sub-samples of each sample, one concentrated, the other dilute, were run on a Durrum D-500 single column microbore ion exchange resin chromatography apparatus to determine amino acid compositions. Analyses of concentrated sub-samples helped to determine content of amino acids rare in the samples.

7.1.1.3 Elemental analysis

Samples of sixth instar brown, and last instar brown, clear, and opaque silk were dried at room temperature and mounted on a carbon stub with graphite glue. Qualitative analyses for elements larger than carbon were conducted with a Cambridge Stereoscan S150 scanning electron microscope, fitted with a Kevex 7000 X-ray analysis unit. Each sample was scanned, the stub 45 degrees to the beam, until the spectrum backgrounds reached the background level obtained from a scan of a bare area on the stub.

7.1.2 Rotation of plane-polarized light

A pad of silk obtained in the laboratory, as described in Chapter 6, and a sample of *S. vittatum* cocoon silk, both mounted unstained in Canada balsam, were examined with a

Carl Zeiss Jena Ampivol polarizing microscope.

7.2 Results

7.2.1 Silk Development and composition

7.2.1.1 Development

Labial glands of *Simulium vittatum* larvae contained silks appearing (Figure 40) brown, clear (both transparent) and opaque (white in reflected light).

A X^2 test for independence of the data in Table I showed that the data were not independent ($p < 0.005$). Thus colour of silk in labial glands was associated with larval maturity. Glands of fourth to sixth instar larvae contained only brown silk (Figure 40a, Table I). In last (seventh) instar larvae of normal thorax size, brown silk filled the gland from bend (be) to exit (arrow) (Figure 40b, 40c). Clear silk filled the closed side of the gland in younger last instar larvae (Figure 40b, Table I), while in more mature larvae, opaque and clear silk filled the closed side (Figure 40c, Table I). In last instar larvae of large thorax size, opaque silk filled the whole gland (Figure 40d), except near the gland exit (Table I). These larvae had darkened respiratory histoblasts and pigmented pupal hooks could be seen through the larval cuticle.

Figure 40. Changes in colour of contents of labial glands of *Simulium vittatum* larvae.

a. sixth instar,

b. early seventh instar,

c. later seventh instar,

d. late seventh instar with enlarged thorax.

Arrow points to salivary meatus.

Scales: a, 200 um; b-d, 500 um.

be- bend in gland,

br- brown silk,

cl- clear silk,

e- closed end of gland,

gl- epithelium of gland,

o- opaque silk.

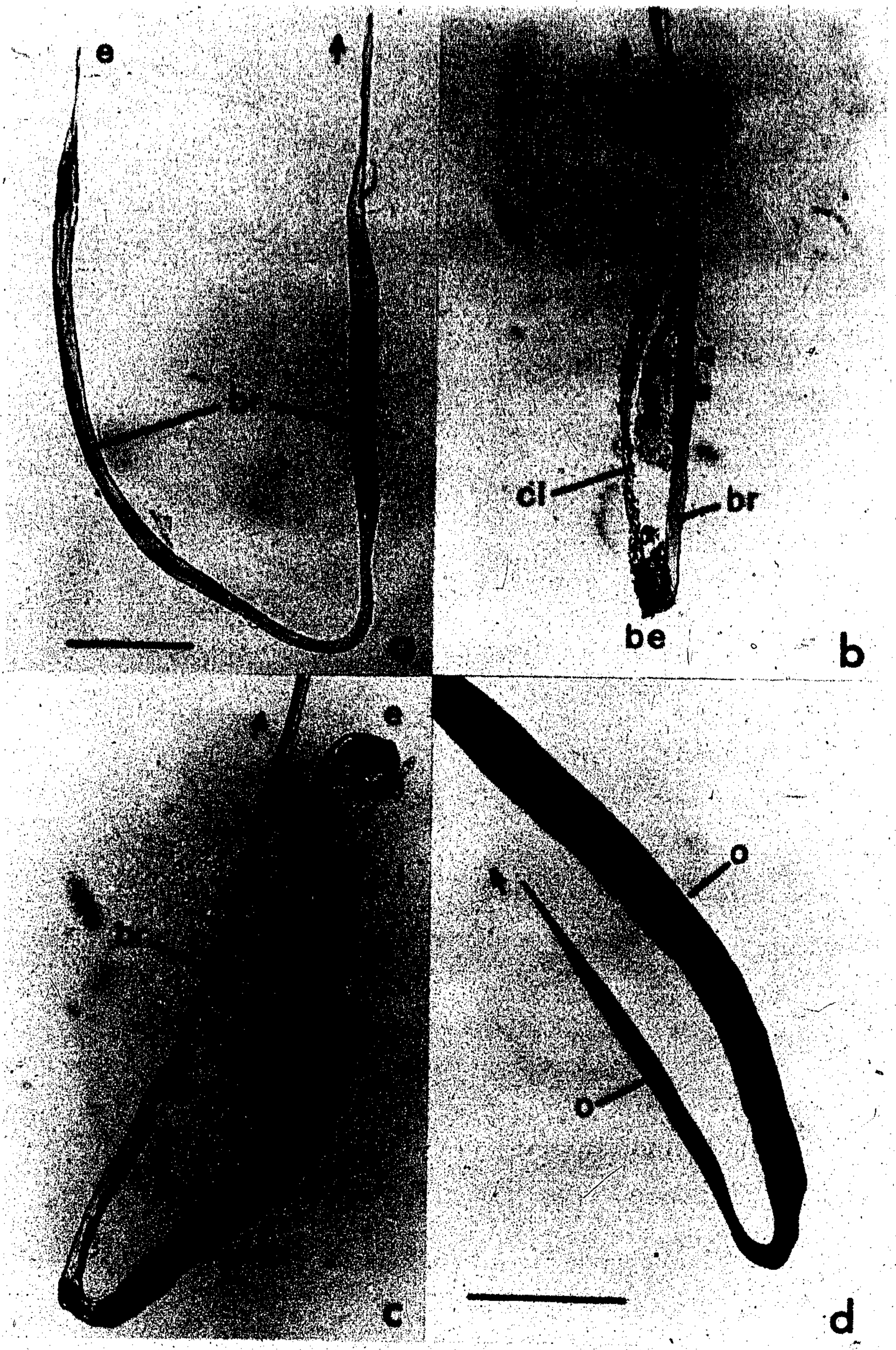

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Table I. Change in colour of silk in labial glands as *Simulium vittatum* larvae mature.

Instar (thorax proportion)	Number of larvae in each class		
	Silk colour		
	Brown only	Clear present	Opaque and brown only
4-6	14	0	1
7 (normal)	0	12	4[1]
7 (large)	0	1	6[2]

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[1] Brown silk filled at least one-half of the length of the gland.

[2] Brown silk filled less than a quarter of the length of the gland, or was not found.

Table II. Amino acid compositions of silks of *Simulium vittatum* larvae (residues per 100 total residues).

Amino acid	Mid-instar brown silk	Last instar brown silk	Clear plus opaque silk
aspartic acid + asparagine	6.8	6.9	7.6
threonine	8.0	8.1	3.1
serine	7.1	6.6	8.2
glutamic acid + glutamine	6.8	6.8	11.2
proline	7.6	7.9	3.3
glycine	34.0	32.2	16.5
alanine	5.7	6.1	11.7
valine	2.5	2.9	2.7
methionine	0.0	0.7	1.4
isoleucine	1.2	1.5	1.5
leucine	2.3	2.4	1.9
tyrosine	1.0	2.0	6.6
phenylalanine	1.1	1.5	1.9
histidine	0.0	0.4	2.4
lysine	12.0	9.2	8.5
arginine	3.7	4.7	11.3
Total	99.8	99.9	99.8

7.2.1.2 Amino acid composition

Brown silks of fourth- to sixth-instar and last-instar larvae had similar amino acid composition. Their composition differed markedly from that of clear plus opaque silk (Table II). Proportions of threonine, proline, and glycine were twice as high, and proportions of alanine, tyrosine, histidine and arginine were half as high in brown silks than in clear plus opaque silk (Table II). The sample of clear and opaque silk containing mostly clear silk differed little in preliminary analysis of amino acid content from the sample of clear plus opaque silk containing mostly opaque silk.

7.2.1.3 Elemental analysis

All samples contained phosphorus, sulphur, potassium, and calcium (Figure 41). Small amounts of sodium and magnesium were in both brown silk samples (Figure 41a, b). Two shoulders beside the phosphorus peaks on the sixth instar trace (Figure 41a) are silicon and aluminum peaks, probably heightened by the high phosphorus and sulphur peaks nearby.

Sixth instar brown silk had the highest levels of phosphorus, sulphur, and potassium (Figure 41a). Last instar brown silk had the highest level of calcium (Figure 41b). Clear silk had the lowest levels of all elements (Figure 41c). Levels of phosphorus and sulphur were almost as high in opaque silk as they were in sixth instar brown silk (Figure 41a, d).

7.2.2 Rotation of plane-polarized light

Silk in the cocoon rotated plane-polarized light, but silk in the pad rotated light very little, if at all.

7.3 Discussion

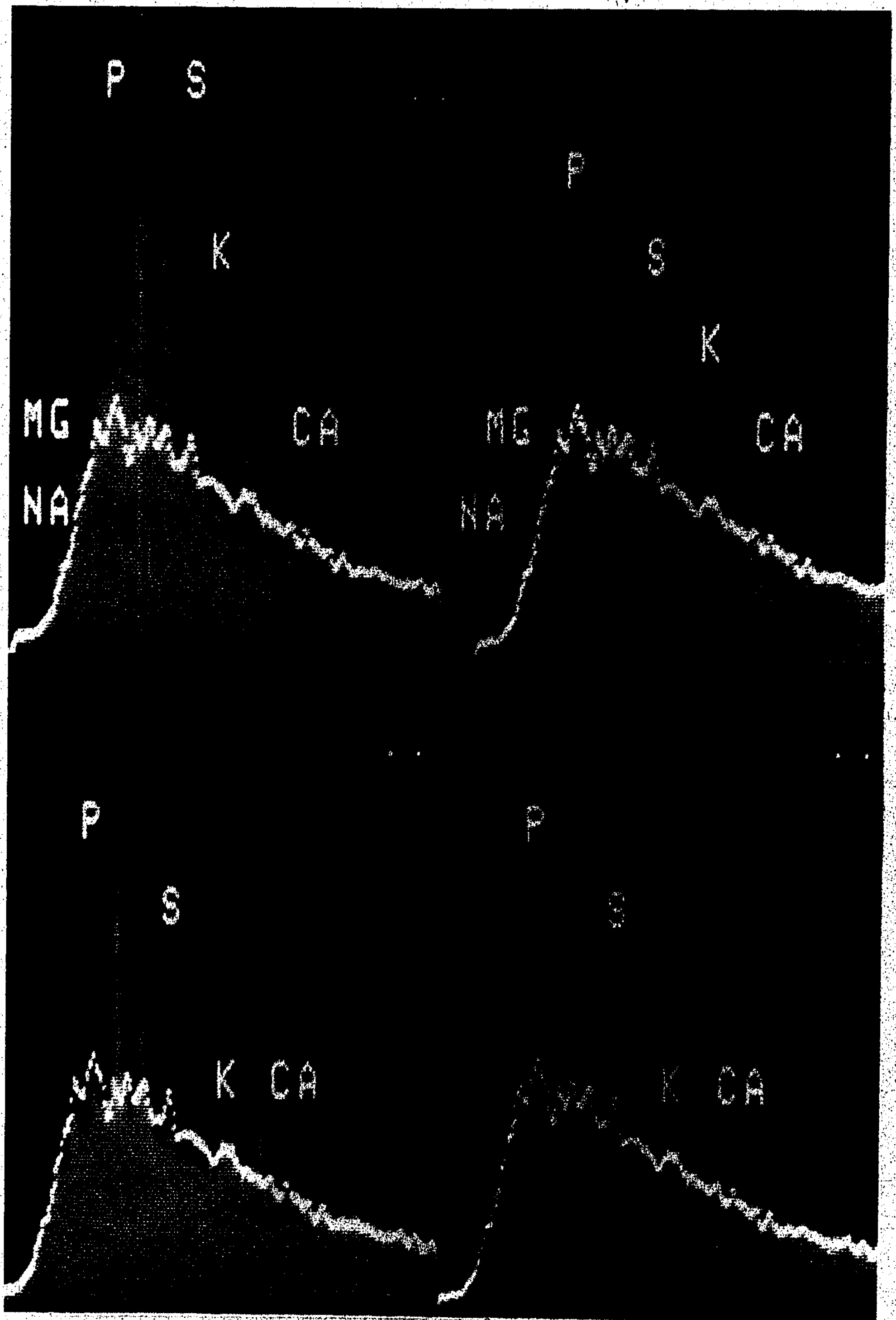
7.3.1 Composition of silk of *Simulium vittatum*

Engster (1976b) reported the amino acid content of unpurified silk of *Pycnopsyche guttifer* (Trichoptera; Limnephilidae). Compared to purified fibroins (Lucas *et al.*, 1960) *S. vittatum* and *P. guttifer* silks have low proportions of alanine, high proportions of the basic amino acids lysine and arginine, and generally high proportions of amino acids with large side chains.

Grossbach (1977) reported amino acid analyses for unfractionated *Chironomus tentans* and *C. pallidivittatus* (Diptera; Chironomidae) silks. These silks are similar to clear plus opaque *Simulium vittatum* silk in amino acid composition, except that the *Chironomus* silks have no tyrosine, much less glycine, and somewhat more lysine. Grossbach (1977) noted the "... high proportion of the basic amino acids lysine and arginine which together account for about 25% of the amino acid residues...".

Figure 41. Relative levels of elements heavier than carbon in contents of labial glands of *Simulium vittatum* larvae.
a. brown silk, sixth instar;
b. brown silk, seventh instar;
c. clear silk, seventh instar;
d. opaque silk, seventh instar.
White trace is background level from scan of bare area on stub.

CA- calcium,
K- potassium,
MG- magnesium,
NA- sodium,
P- phosphorus,
S- sulphur.



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Lucas *et al.* (1960) could not correlate similarity of amino acid content of silks with similarity in biological function of the silks or degree of relatedness of the silk-spinning arthropods they studied. On the other hand, common to the five aquatic silks so far analyzed are high proportions of the basic amino acids lysine and arginine, and fairly high proportions of amino acids with large side chains. As well, *Simulium vittatum*, and *Chironomus tentans* and *C. pallidivittatus* belong to closely related families, and their silks are similar, overall, in amino acid content.

Elemental analysis showed no simple relation between colour and content of elements heavier than carbon. Opaque silk could be polymerized clear silk, or clear silk to which some organic substance has been added. Engster (1976a) suggested that *Pycnopsyche guttifer* labial gland contents changed from translucent to opaque because of alignment of fibroin molecules, polymerization, or tanning.

Brunet and Coles (1974) studied the tanning of Saturniidae (Lepidoptera) cocoon silks. When these white silks are wetted, enzymes and phenols in the silk react to produce, after oxidation, tanning agents which turn the silk brown. Since lysine is so common in the aquatic silks studied, tanning would produce many crosslinks between protein molecules. The crosslinks could bind ribbons of silk where they touch each other, strengthening cocoons especially.

7.3.2 Changes in silk as larvae develop

The close similarity in amino acid content between fourth to sixth instar brown silk and last instar brown silk suggests, but does not prove, that the two have identical protein content, since proportions of amino acids in a sample tell nothing of the number of proteins, their size, or their amino acid sequences.

Simulium vittatum larvae spin a special proteinaceous substance, brown silk, to make attachment discs and anchored lines. As larvae mature, growing imaginal discs enlarge the thorax. This is about a day before cocoon spinning begins (Hinton, 1958). Opaque silk, the only kind found in glands of these very mature last instar larvae, probably makes up cocoons. Clear silk, never reaching the mouthparts, is probably a precursor of opaque silk. Arrival of opaque silk at the mouthparts could stimulate larvae to begin spinning their cocoons.

7.3.3 Mechanical properties

Differences in amino acid content between silks can signal differences in mechanical properties (Wainwright *et al.*, 1976), and differences in abilities of silk in cocoons and pads to rotate light passing through them imply differences in other physical properties.

Larval silk, as attachment pads and anchored lines, resists only tensile stresses from the larvae gripping it. A cocoon, besides resisting tensile stresses pulling it from the substrate, resists compressive stresses. The cocoon, like a broad arch, transmits to the substrate compressive stresses that the pupa would have felt. The matrix that probably suspends the protein fibers probably translates compressive stresses on the cocoon to tensile stresses in the protein fibers. Thus the difference in composition probably reflects different binding properties of the protein fibers to the matrix, rather than a special conformation of the protein for resisting compression.

7.3.4 Further work

Labial glands of black-fly larvae appear to have only one secretory region (MacGregor and Mackie, 1967). Silks these glands produce have well-defined roles and relatively large and clean samples can be obtained. It would be interesting to study how the change of secretion from larval to cocoon silk is controlled, to look for changes in the pattern of puffs on the chromosomes (Basrur, 1959), and to check for changes in fine structure.

Measuring mechanical properties of each silk could also be rewarding.

Preliminary study showed changes in volume and colour of silk in parasitized larvae. Further study of silk production of parasitized larvae could have practical use.

8. Evolution of attachment of black-fly larvae

To the extent that larvae in Simuliidae have the same general structure and follow the same kind of life (Crosskey, 1981), and to the extent that *Simulium vittatum* larvae are typical black-fly larvae, results of the present studies can be applied to Simuliidae as a whole. It would be interesting to find simuliid larvae with very different structures (*e. g.*, lacking tubercles on hooks of anal prolegs), or different behaviour during looping. It would be illuminating to study the reasons for those differences.

Black-fly larvae attach themselves to the substrate by combining, through their behaviour, elements structural and secreted. We would understand how this method of attachment arose if we knew how changes of structure, secretion, and behaviour corresponded to each other, and to invasions of what Bock (1965) called "adaptive zones." The following inferences suggest that the method of attachment of larval black-flies arose through adaptation to feeding on rocks over which water flowed, and that the method was refined during a shift to passive filter feeding.

8.1 Method of inference

Anderson (1979) inferred sequences of evolutionary events by adding information to a reconstructed phylogeny. My method, based on Anderson's (1979) method of evolutionary inference, takes this path:

1) Present a cladogram of the group.

2) Fit character states to the cladogram, and infer phenoclines and intermediate states for these characters. Ex-group comparisons help to guide these inferences.

3) Referring both to the cladogram and to information about the niches of extant species, infer a series of adaptive zones in which the group could have evolved. To fill gaps in the series, infer intermediate adaptive zones.

4) Present the cladogram again, adding information gathered in steps 2 and 3, to show how changes in the group correspond to each other.

5) Follow the lineage through inferred changes of character state and adaptive zone to guess at how the group evolved.

Anderson (1979), faced, in his study group, with many invasions of the same adaptive zones, dealt generally with adaptations to each zone. The following deals with specific invasions and adaptations along one line.

8.2 Cladogram of Culicomorpha

Figure 42 is a cladogram of the culicomorphan families based on Hennig's (1973) reconstructed phylogeny, with information added from Wood (1978) and Crosskey (1969). Rohdendorf (1974, p. 292) placed Orphnephilidea (=Thaumaleidae) outside of Culicomorpha, but admits (Rohdendorf, 1974, p. 58) insufficient knowledge of that family. Otherwise, Rohdendorf's (1974, p. 292) reconstructed phylogeny of Culicomorpha generally agrees with Hennig's (1973) views. According to Wood (1978) black-flies that do not filter-feed as larvae (*Twinnia*, *Gymnopais*, and *Crozetia*), are descendants of various black-flies that filter-fed as larvae. Simuliids with epizoic larvae (living attached to other animals) belong to *Simulium (Phoretomyia)* and to *S. (Lewisellum)*, and are most closely related to *S. (Meilloniellum) hirsutum* Pomeroy (Crosskey, 1969).

8.3 Character states

Table III displays names of characters and shows, with abbreviations, clines in their states, derived states listed after ancestral states. These character states are briefly described in the following four paragraphs.

Lateral palatal brushes of thaumaleid (Leathers, 1924), dixid, and culicid larvae have many rows of short bristles (f). A lateral palatal brush (cephalic fan) of a black-fly larva has a single row of long bristles (f'). Larvae in different simuliid lines have simple lateral palatal brushes, or none at all (f'') (Wood, 1978).

Larvae in most (see Keilin, 1944) dipteran families exchange respiratory gases through spiracles (g). Dixid, culidid, thaumaleid, and some chaoborid larvae belong to that group. Other chaoborid larvae, and larvae of ceratopogonids, chaoborids, and simuliids exchange respiratory gases across the cuticle (g').

Outside Culicomorpha, only nymphomyiid (Cutten and Kevan, 1970) and tanyderid (Exner and Craig, 1976) larvae have anal prolegs. The positions (ventrolateral to the anus) of anal combs on dixid larvae (Peters, 1981), of the anal apparatus on some chaoborid larvae (Peus, 1934), and of the ventral brush on culicid and chaoborid larvae suggest that primitive culicomorph larvae had at least rudimentary anal prolegs (p). No nematoceran larva outside Thaumaleidae, Ceratopogonidae, Chironomidae, and Simuliidae has thoracic (p') prolegs (Hinton, 1955). Simuliid larvae have prolegs with supporting sclerites and hook tubercles (p'').

Hinton (1955) suggested that while prolegs of chironomid and ceratopogonid larvae were homologous, prolegs arose independently in the lines leading to Thaumaleidae and Simuliidae.

Two lines of evidence support Hinton's conclusion. Firstly, thoracic and anal prolegs appear as single

Table III. Some clines of larval features in the evolution of Simuliidae from a pre-thaumaleid ancestor.

	Lateral Palatal Brushes
f	many rows of short bristles
f'	one row of long bristles
f''	reduced or lost
	Gas Exchange
g	spiracular
g'	cuticular
	Prolegs
p	anal
p'	prothoracic and anal
p''	fused, sclerites, hook tubercles
	Labial Gland Secretion
s	mucus holding food particles to mouthparts
s'	attachment silk
s''	attachment silk and cocoon silk

structures in simuliid embryos (Kölliker, 1842; Craig, 1969), suggesting that they arose as single structures. D.A. Craig (pers. comm.) interprets the notch in the tip of the developing thoracic proleg (Craig, 1969) as the presumptive gaps in the circlet of hooks. Secondly, thaumaleid larvae bear uninterrupted rows of hooks on their unpaired prothoracic prolegs (Saunders, 1923). This suggests that unpaired thaumaleid and simuliid prolegs differ in origin from the paired prolegs of ceratopogonid and chironomid larvae.

On the other hand, these four families are thought to be closely related (Figure 42). Also, the prolegs arose from homologous segments (Table 50) and are similar in shape and musculature (Hennig, 1948, 1952, 1973; Hinton, 1955). Paired prothoracic and anal prolegs probably arose early in the Chironomoidea line, and these fused independently in the Thaumaleidae and Simuliidae lines. Unpaired thoracic and anal prolegs probably did not arise early in the Chironomoidea line for two reasons. Firstly, dipteran larvae outside Chironomoidea possessing anal prolegs, nymphomyiids (Cutten and Kevan, 1970) and tanyderids (Exner and Craig, 1976), have paired anal prolegs. Secondly, primitive ceratopogonid (Hennig, 1973) and chironomid (Brundin, 1966) larvae have paired thoracic and anal prolegs while larvae in more derived groups in these families (Hennig, 1973) have fused anal or thoracic prolegs, or both (Table 50).

Hinton (1955) described and illustrated prolegs of some chironomoid larvae, but he showed no attempt to homologize muscles of prolegs. Some of the differences Hinton (1955) saw could be explained by different degrees of fusion. Hinton (1955) realized that differences in degree of fusion should not suggest differences of origin, since he noted (*e. g.*, Table IV) that prolegs of some chironomids and ceratopogonids are variously fused and reduced.

An attempt to homologize muscles and sensilla of prolegs of members of Chironomoidea would help to resolve this question. Until then, Hennig's (1948, 1950, 1973) view still stands: prolegs of chironomoid larvae are probably homologous.

Table IV. Number and distribution of prolegs of chironomoid larvae (from Hinton, 1955).

	Thorax I	Abdomen 9	Abdomen 10
Thaumaleidae	1	1	- - -
Simuliidae	1	1	- - -
Ceratopogonidae	0-2	1-2	- - -
Chironomidae	1-2	- - -	1-2

Labial glands of primitive (Hennig, 1973) ceratopogonid larvae produce silk (s') used for attachment (Thomsen, 1937), as do glands of chironomid and simuliid larvae. Labial-gland secretions of dixid, culicid, chaoborid, or thaumaleid larvae need not wet food since the larvae are aquatic, and if they began to digest food, water flowing about the mouthparts would carry nutrients away. Instead, these secretions would probably be mucus (s), holding particles of food on the mouthparts. Mucuous (from glands other than labial glands) holds particles on mouthparts of larval simuliids (Ross and Craig, 1980). Among culicomorph larvae, only simuliids and perhaps dixids (Elliott and Tullett, 1977; Pennak, 1978, p. 681) produce a second labial gland secretion used for attaching and protecting the pupa (s''). At least some chironomid larvae spin cocoons (Darby, 1962), but whether these are made of a second secretion is unknown.

8.4 Adaptive zones

Early dipteran larvae probably ". . . populated semi-liquid media such as detritus or decaying plant matter . . ." (Rohdendorf, 1974; p. 8). They would have lived half-submerged in these media, keeping their spiracles above the surface. While thaumaleid larvae are hygropetric, (Thienemann, 1909), dixid, culicid, and chaoborid larvae are pelagic. Hennig (1973) suggested that dixid and thaumaleid larvae live at the interface of water and land. Their common ancestor could have lived semi-submerged in a film of water. The semi-submerged zone helps to fill the gap between the early dipteran semi-liquid zone, the pelagic zone, and the hygropetric zone. Detritus, algae, and microorganisms would be abundant in the semi-submerged zone; especially algae because a film of water would filter out little sunlight. Algae makes up part of the diets of culicid (Pucat, 1965), thaumaleid (Stone, 1964), ceratopogonid (Thomsen, 1937), chironomid (Brundin, 1966), and simuliid (Burton, 1973) larvae.

Hygropetric zones, where films of water flow over rocks, would have less detritus and plankton than the semi-submerged zone, but would have flourishing crops of encrusting algae. Riffles and splashes would make hiding from predators easier than in the semi-submerged zone.

Below the surface of flowing water, in the benthic (lotic) zone, algae do not grow as well as near the surface, but detritus collects where the water slows down.

Animals in the passive filter feeding zone are independent of strictly local food sources in that their food can come from sources some distance upstream.

The epilithic zone fills the gap between the benthic and the passive filter-feeding zones. The strong currents in this zone orient animals and carry many particles.

Larvae along the simuliid line last invaded the epizoic zone. Hosts probably protect larvae and pupae from some predators, from strong currents (Lewis, 1960), and from droughts (Raybould *et al.*, 1978), but larvae must compete with others of their own species for attachment sites (Grenier *et al.*, 1965). Since larvae in non-phoretic species live in the same streams that phoretic larvae live in (Grenier *et al.*, 1965), intraspecific, rather than interspecific, differential survival probably drove the simuliid invasion of the epizoic adaptive zone.

8.5 Evolution of larval attachment

Figure 42 shows how changes of larval features could have corresponded to changes of larval adaptive zone in the ancestry of Simuliidae. According to this scheme, larvae in the semi-submerged adaptive zone breathed through spiracles (g), had rudimentary anal prolegs (p), scraped particles up from the substrate, and filtered them out with lateral palatal brushes (f). Lines in the semi-submerged zone developed the mandible-past-hypostomium method of scraping

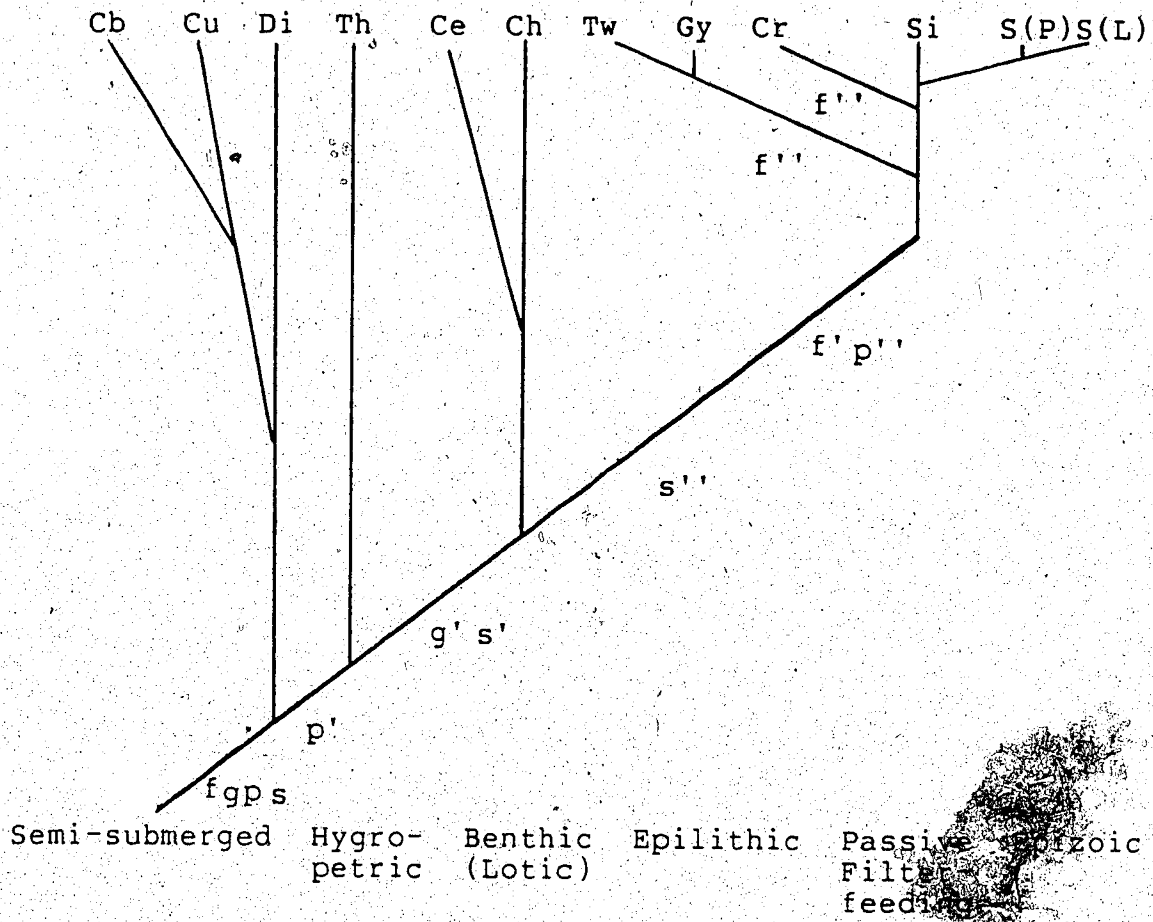

Figure 42. A cladogram of Culicomorpha, based on Hennig (1973), Wood (1978), and Crosskey (1969), showing adaptive zones invaded (bottom line), and changes of some features (see Table III) of larvae. Cb=Chaoboridae, Ce=Ceratopogonidae, Ch=Chironomidae, Cr=*Crozetia*, Cu=Culicidae, Di=Dixidae, Gy=*Gymnopais*, Si=most Simuliidae, S(L)=*Simulium* (*Lewisellum*), S(P)=*S.* (*Phoretomyia*), Th=Thaumaleidae, Tw=*Twinnia*.

the bottom (Chapter 3), and various brushes on the mouthparts for catching and concentrating floating particulate food. Food particles stuck to mouthparts coated with mucuous from the labial glands (s). Lateral bendings of their worm-like bodies allowed these larvae to move about without having to work against surface tension (Chapter 2).

Larvae in one descendant line specialized in filtering planktonic food, and became able to float. This line gave rise to Dixidae, Culicidae, and Chaoboridae.

Larvae in a sister descendant line specialized on grazing encrusted food, and became able to grasp the substrate with setae ventro-lateral on the prothoracic cuticle (p'). More firmly anchored, these larvae could graze more efficiently in the semi-submerged zone, and were even able to graze while water flowed around them. This line entered the hygropetric zone, developed this thoracic proleg (p') further, and gave rise to Thaumaleidae.

Larvae in a sister line to Thaumaleidae could have laid mucus from their salivary glands down on the substrate then retrieved the mucus and the food stuck to it as some chironomid larvae now do (Walshe, 1951). Descendants could have left mucous behind on the substrate for the thoracic and anal prolegs to grasp. Descendant larvae that could rely on labial gland silk (s') alone for attachment, not needing a mat of algae to grasp, could scrape the substrate clean of algae. The mandible-past-hypostomium method of scraping the substrate became the method of cutting off silk (Chapter 3), and some brushes on the mouthparts that once handled food, came to handle silk.

Improved attachment that labial gland silk provided allowed development of cuticular gas exchange (g'). Cuticular gas exchange is adequate for an aquatic insect's needs only if water flows over the cuticle (*e. g.* Wiggins, 1977, pp. 16-19). Silk, enabling larvae to stay attached in flowing water, allowed them to live free of direct dependence on atmospheric air, in flowing water at any depth. This line entered the benthic zone of running (lotic) water, and gave rise to Ceratopogonidae and Chironomidae. Larvae in ceratopogonid and chironomid lines later invaded many other adaptive zones, but their primitive adaptive zone was benthic (lotic) (Brundin, 1966; Thomsen, 1937).

Rheophilic larvae in a sister line to Ceratopogonidae plus Chironomidae attached themselves to the substrate by anal prolegs, and trailed their bodies downstream. Larvae in this line entered the epilithic zone, where lateral palatal brushes caught, before they washed away, food particles scraped up by the mandibles. Lateral bending for locomotion in the semi-submerged zone became looping in the slower water close to the substrate in the epilithic zone. These larvae spun cocoons of a second labial silk (s''), and molted to pupae inside. Cocoons protected pupae from being washed away, and from being preyed on.

Larvae in a descendant line seldom scraped the bottom, but kept on filtering the passing water. Having entered the passive filter feeding zone, larvae with large head fans, (f') and larvae that held these fans in faster currents above the boundary layer were selected for. This in turn caused larvae with strong, efficient attachment to be selected for. Prolegs fused and developed special sclerites; anal prolegs shortened and hooks developed tubercles (p''). Attachment would not have improved greatly without selection pressure caused by improved filtering faculties. At the same time, filtering could not have improved greatly without matching improvements in attachment.

Black-fly larvae at headwaters have few upstream sources of floating food particles. Ancestors of *Twinnia* and *Gymnopais*, depending more and more on food they scraped up with their mandibles and hypostomial teeth, retained until the pupal stage, the fanless condition (f'') of first-instar larvae of their *Prosimulium* forebears (Wood, 1978). Ancestors of *Crozetia* raked in extra food by touching the substrate with their fan tips and then closing their fans. Tips of fan rays in that line became rakes and the closing mechanism simpler (see Davies, 1974). Larvae in these lines have reinvaded the epilithic and the benthic (lotic) adaptive zones, and weaker detachment forces on larvae there could help to explain the loss of posterior arms of anal sclerites on *Twinnia* and *Gymnopais* larvae.

Larvae in another line of Simuliidae (Crosskey, 1969) attached themselves to river crabs (*Simulium* (*Lewisellum*)), and to mayfly nymphs (*S.* (*Phoretomyia*)). Some changes of larval structure (Crosskey, 1969), inconsistent among the species, have occurred in this epizoic zone.

While the form of attachment of black-fly larvae developed through a continuum of adaptive zones, it also developed through preadaptations. Lateral bending, adapted for locomotion in films of water, was preadapted for looping in the slow current near the bottom (Chapter 2). Labial glands, adapted for producing saliva, were preadapted for producing mucus which stuck food particles to mouthparts in the semi-submerged and hygropetric zones. This preadapted them further for producing silk grasped by the prolegs for attachment. This preadapted labial glands still further for producing silk spun into cocoons that attached and protected pupae. Hooks on both thoracic and anal prolegs, adapted for grasping the substrate and encrusting algae, were preadapted for grasping silk. Brushes on the mouthparts, adapted for concentrating particles and handling filaments of algae, and for spreading mucus amongst each other, were preadapted for spreading silk on the substrate. Mandibular and mental teeth, adapted for working together to scrape up encrusting layers of algae and detritus, were preadapted for cutting filaments of silk.

8.6 Summary

To summarize, Culicomorpha probably came to be when ancestors entered, as larvae, the semi-submerged adaptive ne. Culicomorph larvae in the line leading to Simuliidae invaded, in succession, at least four adaptive zones. At least two independent reversions to the benthic (lotic) zone occurred, and two diverging lines invaded the epizoic zone.

Faculties developed in one adaptive zone allowed invasion of new adaptive zones adjacent, and did not prevent re-invasion of at least one adaptive zone.

The method of attachment unique to black-fly larvae probably arose through adaptation to feeding in flowing water, and was probably refined during a shift to epilithic life and passive filter feeding. Inferred developments in adaptive zones invaded in the sequence: semi-submerged, hygropetric, benthic (lotic), epilithic, and passive filter feeding give rise to this conclusion.

This picture, consistent with available information on niches of living culicomorph larvae, and consistent with views now held on culicomorphan phylogeny, shows how some changes of structure, secretion, and behaviour, could have corresponded to each other and to invasions of adaptive zones. Through this sequence of developments, larvae were probably preadapted for looping, for silk production, for prolegs grasping silk, and for mouthparts handling and cutting silk. New views emerged of the niches and anatomy of larvae ancestral to Culicomorpha and of larvae ancestral to Simuliidae. Improved attachment probably allowed development of cuticular gas exchange in culicomorphs, and of passive filter feeding in Simuliidae.

9. Conclusion

To attach themselves to the substrate, *Simulium vittatum* larvae combine, through coordinated behaviour, sophisticated structures with a specific secretion.

Behaviour for attachment follows a specific path. Closing its cephalic fans, and bending its body laterally, a *S. vittatum* larva brings its head to the substrate. It grasps the free end of the ribbon of silk at the salivary slit with lacinial spines and external maxillary brushes, and protracting the maxillae, applies that silk to the substrate. The thoracic proleg attaches to the silk, and the mouthparts trail a bit of silk on ahead. Anterior to where the thoracic proleg is attached, the larva spins out more silk on the substrate. The maxillae daub and spread silk over a wide area.

When the area of substrate is covered, the larva makes the ribbon of silk narrow by moving its salivary slit posteroventrally. Adducting its mandibles, the larva cuts the silk between the mandibular and mental teeth. The larva then abducts its mandibles, and plants its mental teeth in the silk at the anterior end of the new pad. Mandibles then adduct nearly to the mentum, gathering and grasping silk to attach the head to the pad.

The larva then detaches its anal proleg from the old pad of silk. Bending its body laterally again, it brings its anal proleg to the pad of silk between the attached thoracic proleg and mouthparts. Anterior hooks of the anal proleg attach first, then the anal sclerite, flexed inward, straightens, pulling posterior hooks into the silk.

The larva then abducts its mandibles, and its head releases from the new pad. Almost at the same time, the larva retracts the centre of the tip of its thoracic proleg, detaching central hooks of the thoracic proleg from the pad. Hooks peripheral on the thoracic proleg detach last from the fresh sticky silk, bristles of the lateral sclerite pushing the silk and hooks apart. The attachment is complete. Tubercles on hooks of the anal proleg prevent those hooks from working loose from the pad.

Larvae do not scrape up the pads of silk they abandon, and these pads bear marks of where the thoracic proleg, mouthparts and the anal proleg attached. Pads of silk larvae attach to while moving about contain less silk than pads larvae attach to while filter-feeding. This probably helps larvae to conserve silk. Pads of silk rot away in a few days, so a larva risks detachment if it remains attached to a pad for too long.

When larvae approach the molt to pupa, their labial glands stop secreting the silk that larvae use for attachment. While larvae are depleting their stores of this silk, cells of labial glands secrete a different silk that larvae later spin to make cocoons.

Elements of the simuliid method of attachment could have arisen in the following way. Anal prolegs and lateral bending helped larvae to move about in films of water covering solid substrates, the ancestral culicomorphan habitat. Prothoracic prolegs, arising later, freed the head from its former role as the only anterior attachment, and made feeding easier in flowing films of water, the ancestral chironomoidean habitat. Attachment of hooks of prolegs to viscous, sticky saliva (silk) helped larvae in the benthic (lotic) zone to stay attached to the substrate where algae were scarce. Finally, changes to prolegs helped larvae in the epilithic zone to attach strongly to silk and thus stay attached while passively filter-feeding.

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11. Appendix

Table A-1. Tentative homologies for structures of larval labropalata, of taxa related to Simuliidae.

Term used here Simuliidae	Exner and Craig, 1976 Tanyderidae	Harbach and Knight, 1980 Culicidae	Saether, 1976 Chironomidae
cephalic fan	lateral palatal brush	lateral palatal brush	chaetae of labrum (or) spinulae (or both)
cephalic fan stem	---	---	---
labropalatum	labrum (plus) epipharynx	labropalatum	labrum (plus) palatum
anterior labropalatal brush (apb)	---	anteromedian palatal brush	lamella of labrum
mid-labropalatal brush (mp)	messor	(part of) midpalatal brush	f
bristles of mid-labropalatal brush	f	midpalatal brush filament	chaetulae laterales of palatum
median labropalatal brush (mb)	---	(part of) midpalatal brush	pecten epipharyngis
bristles of median labropalatal brush	---	---	f
labropalatal comb (lc)	epipharyngeal sensilla	(part of) midpalatal brush and seta 3L-P	ungula
posterior labropalatal brush (pp)	---	(part of) midpalatal brush	chaetulae basales of palatum

f=figured, but not named.

Table A-2. Terms for structures of the simuliid larval labropalatum.

Term used here	Puri, 1925	Chance, 1970	Craig, 1977	Peterson, 1977
cephalic fan	(part of) cephalic fan	cephalic fan	cephalic fan	cephalic fan
cephalic fan stem	(part of) cephalic fan	cephalic fan stem	fan stem	stalk of cephalic fan
labropalatum	labrum and epipharynx	labrum	labropalatum	labrum
anterior labropalatal brush (apb)	f	dorsal brush	f	f
mid-labropalatal brush (mp)	f	main bristled area	(part of) anterior palatal brush	f
bristles of mid-labropalatal brush	long simple setae	groups of 2 to 4 short bristles	---	---
median labropalatal brush (mb)	elongated patch	spindle-shaped patch, (part of) main bristled area	(part of) anterior palatal brush	(part of) medioventral setae
bristles of median labropalatal brush	hooks (and) hooklets	conical, spine-like bristles	---	---
labropalatal comb (lc)	tooth-like projections	teeth of labral sclerite	f	(part of) medioventral setae
posterior labropalatal brush (pp)	long branching setae	f (on) ventral lobe	(part of anterior palatal brush	---

f=figured, but not named.

Table A-3. Tentative homologies for structures of larval maxillae, of taxa related to Simuliidae.

Term used here Simuliidae	Exner and Craig, 1976 Tanyderidae	Kramer, 1954 Ptychopteridae	Harbach and Knight, 1980 Culicidae	Saether, 1976 Chironomidae
maxillary palpus	maxillary palp	maxillary palpus	maxillary palpus	maxillary palp
palpifer	palpiger (and) palpifer	f	merostipes	palpiger
palpifer brush	f	---	---	tetrahedral lamelles
galea	galea	(part of) galea-lacinial complex	galeastipes	(part of) galea
sculptured area	adoral molar region	---	---	---
apicodorsal sensillum (ds)	(part of) adoral sunken brush	---	(part of) seta 1-Mx	paraxial seta and sensilla basiconica
apicoventral sensilla	2 large curved sensilla	---	(part of) seta 1-Mx	antaxial seta and sensilla basiconica
dorsal brush	---	---	maxillary pilose area	(part of) lamelles
apical brush	(part of) adoral sunken brush	(part of) circular setae	(part of) maxillary brush	(part of) lamelles
medial brush	(part of) adoral sunken brush	(part of) circular setae	(part of) maxillary brush	pecten galearis

f=figured, but not named.

Continued on next page.

Table A-3 (concluded). Tentative homologies for structures of larval maxillae, of taxa related to Simuliidae.

Term used here Simuliidae	Exner and Craig, 1976 Tanyderidae	Kramer, 1954 Ptychopteridae	Harbach and Knight, 1980 Culicidae	Saether 1976 Chironomidae
aboral brush (bb)	---	vertical setae	laciniarastrum 1	---
ventral aboral brush (vb)	(part of) cardo brush	---	(part of) laciniarastrum 1 or 2?	---
lacinia (la)	lacinia	(part of) galea-lacinial complex	laciniastipes	(part of) galea
lacinial teeth (tl)	---	---	laciniarastrum 2	(?) appendix seta
ventral adoral brush (va)	(part of) cardo brush	f	laciniarastrum 3	anterior cheatae.

f=figured, but not named.

Table A-4. Terms for structures of the simuliid larval maxilla.

Term used here	Chance, 1970	Crosby, 1974	Craig, 1977
maxillary palpus	palp of maxilla	maxillary palp	maxillary palpus
palpifer	cardo	cardo	palpifer
palpifer brush	f	outer dorsal hair tuft of cardo	f
galea	galea	galea	(part of) maxillary lobe
sculptured area	lobulate area	---	sculptured area
apicodorsal sensillum (ds)	---	sensory spine (and) associated sensory hair	basiconic sensillum and trichoid sensillum (and) distal sensilla
apicoventral sensillum	spine	---	basiconic sensillum and trichoid sensillum
dorsal brush	diffuse brush	---	dorsal brush
apical brush	apical brush	f	(part of) apical brush
median brush	middle brush	---	(part of) apical brush
aboral brush (bb)	large oral brush	f	aboral apical brush
ventral aboral brush (vb)	f	---	---
lacinia (la)	lacinia	lacinia	(part of) maxillary lobe
lacinial teeth (tl)	teeth	teeth of lacinia	lacinial bristles
ventral adoral brush (va)	small oral brush	---	f

f=figured, but not named.

Table A-5. Tentative homologies for structures of larval labiohypopharynxes, of taxa related to Simuliidae.

Term used here Simuliidae	Anthon, 1943 Trichoceridae and Ptychopteridae[1]	Kramer, 1954 Ptychopteridae	Harbach and Knight, 1980 Culicidae	Saether, 1971 Chironomidae
labiohypopharynx	f	labiohypopharyngeal complex	labiohypopharynx	prementohypopharyngeal complex
hypopharynx (hx)	Hypopharynx	hypopharynx	hypopharynx	hypopharyngeal lobe (and) hypopharynx
hypopharyngeal brush	f	---	---	pecten hypopharyngis
rows of hypopharyngeal brush (r1-r3)	f (5 rows)[2] (many rows)[3]	---	---	(many rows of) hypopharyngeal scales
salivary meatus (x)	f	salivary duct opening	salivary meatus	---
prelabium	f	labium	prelabium	prementum
ligula	Glossa, Paraglossa	---	ligula	ligula (plus) paraglossa
labial palpus (lp)	Labialtaster	*labial palpus*	*labial palpus*	sensillar 1?
sensilla of labial palpus (S1-S5)	---	---	setae 1-Lh to 6-Lh	sensillar 1-5 of labium

f=figured, but not named.
[1] Also Psychodidae and Anisopodidae.
[2] *Trichocera*.
[3] *Ptychoptera*.

Continued on next page.

Table A-5 (concluded). Tentative homologies for structures of larval labiohypopharynxes, of taxa related to Simuliidae.

Term used here	Anthon, 1943	Kramer, 1954	Harbach and Knight, 1980	Saether, 1971
Simuliidae	Trichoceridae and Ptychopteridae[1]	Ptychopteridae	Culicidae	Chironomidae
prementum	Praementum	f	prementum	f
dorsal premental brush (dp)	---	---	premental malae	curved scales
lateral premental brushes (pb)	---	f	lateral premental spicules	chaetoids
ventral premental brush (v)	f (on) Ein dem Mentum entsprechendes Areal des Labiums	f?	ventral premental spicules	lamelles

f=figured, but not named.

[1] Also Psychodidae and Anisopodidae.

Table A-6. Terms for structures of the simuliid larval labiohypopharynx.

Term used here	Grenier, 1949	Chance, 1970	Crosby, 1974	Craig, 1977
labiohypopharynx	f	labio-hypopharyngeal complex	labiohypopharyngeal complex	labiohypopharyngeal complex
hypopharynx (hx)	hypopharynx	hypopharyngeal lobe	hypopharyngeal lobe	hypopharynx
hypopharyngeal brush	épines bordant l' orifice d' emission de la soie	f	f	dichotomous bristles
rows of hypopharyngeal brush (r1-r3)	trois rangés ... de ... épines	2 rows of bristles	f (one row)	f (2 rows)
salivary meatus (x)	l' orifice de sortie de la soie	(part of) silk canal	opening of silk canal	salivary duct opening
prelabium	labium	labial lobe	labial lobe	f
ligula	lobes internes de la ligula	ligular lobes (or) conical spine-like bristles	ligula lobes	ligular plates
labial palpus (lp)	f	sensory lobes	sensory lobe	labial palpus
sensilla of labial palpus (S1-S5)	sensilles basiconiques du prementum	basiconic sensilla[1]	2 inner and 3 outer sensilla basiconica	5 sensilla: 4 cone-shaped; 1 globular
prementum	prementum (plus) mentum	f	f	f
dorsal premental brush (dp)	épines puissantes	f	f	f
lateral premental brushes (pb)	f	labial brush	labial brush	(part of) labial brushes
ventral premental brush (v)	f	ventral brush	ventral brush	(part of) labial brushes

f=figured, but not named.

[1] *Simulium* spp., 6-8 sensilla; *Cnephia dacotensis*, 6; *Prosimulium* spp., 5.

Table A-7. Tentative homologies for structures of larval postlabia, of taxa related to Simuliidae.

Term used here Simuliidae	Anthon, 1943 Trichoceridae and Ptychopteridae[1]	Harbach and Knight, 1980 Culicidae	Saether, 1971 Chironomidae
mentum (m)	Hypostomium	mentum	mentum (and part of) postmentum
dorsomentum	---	dorsomentum	dorsomentum
flange (fl)	---	---	---
mental teeth	f[2]	dorsomental teeth	mental teeth
median tooth (mt)	f[2]	f	one or two median teeth
intermediate teeth (it1-it3)	f[2]	f	1st to 3rd lateral teeth
corner teeth (ct)	f[2]	?f	4th lateral teeth
lateral teeth (lt)	---	---	5th and 6th lateral teeth
ventromentum (vm)	---	ventromentum	ventromentum
lateral serrations (sr)	---	---	f (on) ventromental plates
submentum	Hypostomalbrücke (and) Postgena	submentum	submentum
submental cleft	---	---	---

f=figured, but not named.

[1] Also Psychodidae and Anisopodidae.

[2] *Ptychoptera* only.

Table A-8. Terms for structures of the simuliid larval postlabium.

Term used here	Crosskey, 1960 and 1969	Craig, 1969	Peterson, 1970 and 1977	Crosby, 1974
mentum (m)	hypostomium	---	hypostomium	mentum (and) mental plate
dorsomentum	f	---	f	f
flange (fl)	f	---	flange	---
mental teeth	apical teeth	mental teeth	hypostomial teeth	mental teeth
median tooth (mt)	median tooth	f	median tooth	median tooth
intermediate teeth (it1-it3)	intermediate teeth	---	sublateral teeth	intermediate teeth
corner tooth (ct)	corner tooth	f	outer lateral tooth	corner tooth
lateral teeth (lt)	(part of) lateral serrations	---	f	lateral teeth
ventromentum (vm)	f	---	f	anterior extension of mentum
lateral serrations (sr)	(part of) lateral serrations	---	f	f
submentum	postgenal bridge	submental in origin	postgenal bridge	submental bridge
submental cleft	postgenal cleft	submental cleft	postgenal cleft	submental cleft

f=figured, but not named.

Table A-9. Terms for structures of the simuliid larval abdomen.

Term used here	Headlee, 1906	Puri, 1925	Grenier, 1949	Crosskey, 1960 and 1969	Rubtzov, 1964	Peterson, 1977
ninth abdominal segment	f (part of last abdominal segment)	(part of) 8th abdominal segment	f	last abdominal segment	[illegible]	(part of) segment VIII
ventral papilla (vp)	---	ventral papilla	f	ventral papilla	---	ventral papilla or tubercle
anal organ (ao)	blood gill	anal gill	,,branchie'' rectale	rectal gill	Rect[illegible]anhang	rectal gill (and) anal gill
posterior proleg	caudal sucker	posterior sucker (and) appendage	appareil postérieur (and) pseudopode	posterior circlet (and) pseudopod	hinteren Haftorgan	f
scales of anal sclerite (sc)	---	simple scales	---	---	kleine Dörnchen	---
anal sclerite	X-shaped piece	X-shaped sclerite	sclérite en X	anal sclerite	Chitenrahmens	anal sclerite
sensilla of anal sclerite (as)	---	f	f	---	Haken des Chitenrahmens	---
laterodorsal expansion (lx)	---	f	expansion latero-dorsale	swollen area of last abdominal segment	Seitenästen des Chitenraumes	f
circlet of hooks	---	circle	couronne	posterior circlet	Ringes	posterior circlet
hooks (ho)	---	hooks	crochets	hooks (and) hooklets	Haken	hooks

f=figured, but not named.

Table A-10. Characteristics of field site on Sturgeon River

Location	100m upstream of bridge on road between Onoway and Calahoo, 25 km NW of Edmonton, Alberta, Canada 114° 02' W, 53° 44' N
Altitude	680 m
Geological Formation	Edmonton, "...composed of gray interbedded bentonitic shales and sandstones..." (Lindsay *et al.*, 1968, p. 18)
Soil	Podzolic (Orthic Gray Wooded)[1]
Phytogeographic Region	Parkland, near transition to Boreal Forest[1]
Dominant Plants	Aspen (*Populus tremuloides* Michaux), and Balsam Poplar (*Populus balsamifera* L.)
Human Economy	Mixed farming

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[1] Lindsay *et al.*, 1968.

Table A-11. Physical characteristics of Sturgeon River at field site, 25 July to 02 August 1979.

Drainage System	Hudson Bay
Sources	Consecutively, Isle Lake, Lac Ste. Anne, Matchayau Lake (6 km upstream), Toad Creek (5 km upstream)
Slope	1/4 m per km
Ice-Free Period	200-220 days, Mid-April to Late October[1]
Substrate	Odd boulders, stones less than 20 cm diameter, on closely-packed gravel "...mainly of quartzites and cherts with occasional arkose pebbles..." (Lindsay *et al.*, 1968, p. 19)
Discharge	1.85-5.66 m³/sec, mean daily[1]
Width	37 m
Maximum depth	70 cm
Velocity	20-30 cm/sec among stakes†; 35-45 cm/sec at polyethylene tape†
Temperature	17-22 °C

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[1] Environment Canada hydrographid station near Villeneuve, below confluence of Sturgeon River with Rivière qui Barre.

†Measured with a Stevens midget current meter.

Table A-12. Chemical and physical characteristics of water in the Sturgeon River at field site, 31 July 1979.

Alkalinity as calcium carbonate:	
Phenolphthalein	0 mg/l
Total	137.6 mg/l
pH	7.18
Conductance	270 umhos
Total residue	215.0 mg/l
Iron	0.14 mg/l
Chloride	4.13 mg/l
Colour	105
Turbidity	0.9 JTU
Sulphate	11 mg/l
Hardness as calcium carbonate:	
Calcium	84 mg/l
Total	148 mg/l

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Table A-12. Concluded. Chemical and physical characteristics of water in the Sturgeon River at field site, 31 July 1979.

Silica	2.65 mg/l
Phosphate:	
Ortho	0.23 mg/l
Meta±Poly	0.07 mg/l
Organic	0.19 mg/l
Total	0.49 mg/l
Nitrogen:	
Ammonia	0.010 mg/l
Organic	1.20 mg/l
Nitrate	0.09 mg/l
Nitrite	0.002 mg/l
Total Kjeldahl	1.21 mg/l
Sodium	22 mg/l
Potassium	5 mg/l
Dissolved oxygen	10.2 mg/l

Table A-13. Biological characteristics of Sturgeon River at field site, 25 July to 02 August 1979.

Dominant Plant	*Potamogeton richardsonii* (Bennett) Rydberg
Aquatic Animals	*Physa* sp.; *Gammarus lacustris* Sars; *Baetis* two spp.; *Cheumatopsyche* sp.; *Hydropsyche* sp.; *Helicopsyche borealis* (Hagen); *Oeetis inconspicua* (Walker); *Ceraclea ariellies* (Denning); *Simulium vittatum* Zetterstedt; *S. venustum* Say or *S. verecundum* Stone and Jamnback‡

‡Few specimens found.

12. Autobiography

I was born in Vermilion, Alberta in 1953, and grew up on my parents's farm a few miles away. There, a respect for animals replaced my initial fears of them. There, for a time, and with my parents's encouragement, I collected butterflies and moths. Small creatures, fairy shrimps, hover-flies, lacewings, horsehair worms, and their small-scale worlds, fascinated me.

I first entered the University of Alberta in 1971, planning to become an environmental-biologist. During a break for work and travel, I remembered the small-scale worlds.

I returned to the University of Alberta in 1974 to become an entomologist. A summer's work on the Athabasca River black-fly project, and a second summer's work on susceptibility of black-fly larvae to insecticide introduced me to black-fly larvae. I received my Bachelor's degree in 1978 and immediately began this project.