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# Population dynamics and intraspecific interactions of an ectosymbiotic midge in a river in southern Maine, USA

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*Abstract.* Ectosymbioses between the Chironomidae and their hosts continue to be documented, but life-history details are not well known. I investigated population dynamics of the commensal midge *Nanocladius* (*Plecopteracoluthus*) sp. #5 over a 3-y period to understand the importance of intraspecific interactions in determining host and attachment site use. Patterns of infestation intensity and prevalence on hosts indicated a peak spring emergence, though emergence extended throughout the open-water period. There was no difference in emergence timing of adults, indicating population synchrony. Temperature played a key role in directing life-history events. As temperature increased in the spring, midges migrated to the dorsal thorax for pupation and this migration coincided with an increase in aggressive behavior by resident midges. The density distribution on hosts suggested these midges detected and attached to hosts in a random manner, but that territorial behaviors and age were important in determining tube-attachment position following host location. The largest midges invariably occurred on the ventral thorax of hosts harboring multiple commensals. In addition, if a midge occurred singly on a host, regardless of instar, it was positioned most anterior on hosts, indicating attachment position was not age-specific. Midges exhibited some ability to discriminate suitable hosts, but further investigations into the cues used by ectosymbionts to detect potential hosts and appropriate-age hosts are warranted.

*Key words:* ectosymbiosis, Chironomidae, Megaloptera, prevalence, infestation intensity.

Commensalisms have received less attention from ecologists than either parasitic or mutualistic interactions (Bronstein 1994). Yet, commensalisms and other *harmless* symbioses may be the most common interactions in multispecies assemblages. For example, Dodds (1997) suggested amensalisms and commensalisms should dominate large interaction webs, even though ecologists focus on competition and predation. The Chironomidae, among aquatic insects, have evolved symbiotic interactions ranging from facultative phoresy to obligate parasitism (reviewed by Tokeshi 1993, 1995), and commensal interactions dominate the known symbioses between midges and their hosts. Twenty-five (69.4%) of the 36 midge species listed by Tokeshi (1993) as forming symbioses with other aquatic organisms are considered commensal. The proportion of known commensal ectosymbionts increases to 75% (21 of 28) if only those species associated with other *insect* hosts are considered. Yet, very little is known of the population biology of these symbionts or the intimacy of their relationships with their hosts.

Bottorff and Knight (1987) provided an exhaustive examination of the ectosymbiotic relationship between the midge *Nanocladius downesi* Steffan (Diptera:Chironomidae) and its stonefly host, *Acroneuria abnormis* Newman (Plecoptera: Perlidae) in a Michigan, USA stream. They showed that this midge occurred attached to its host in all seasons and 4th-instar larvae dominated the population at all times. Further, they suggested that the seasonal and spatial patterns of midges on hosts indicated intraspecific competition among symbionts. Pennuto (1998, 2000) also invoked intraspecific competition as a mechanism to explain emergence patterns observed in a laboratory population of a related species, *Nanocladius* (*Plecopteracoluthus*) sp. #5, attached to host saw-combed fishflies, *Nigronia serricornis* Say (Megaloptera:Corydalidae), in Maine. However, neither Bottorff and Knight (1987) nor Pennuto (1998, 2000) performed behavioral trials to support contentions that midge distribution patterns on hosts resulted from competition.

Several works have shown the frequency distribution of ectosymbiotic midges on their hosts

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does not follow Poisson expectations, either throughout the year or just on some dates (Svensson 1976, 1980, Bottorff and Knight 1987, Jacobsen 1995, 1999, Pennuto 1998, Pennuto et al. 2002). The general interpretation from these analyses is that distribution patterns that are overly uniform or clumped on hosts provide evidence of intraspecific competition, or at least evidence of some biological phenomenon other than chance alone. In contrast, Tokeshi (1986) found the infestation intensity of the commensal midge *Epoicocladius flavens* (Malloch) on mayflies did not differ from Poisson expectations if host year-classes were analyzed separately. He suggested that multiple factors, including environmental stochasticity, midge searching behavior, or size variation of hosts likely were responsible for the frequency patterns observed.

In southern Maine, the midge *N.* (*P.*) sp. #5 is a common, obligate ectosymbiont of *N. serricornis* (Pennuto 1997, Pennuto et al. 2002), occurring on >70% of host individuals in many populations. This midge has neither been observed free-living nor attached to any other host in Maine (Pennuto et al. 2002). Host fishflies are long-lived, with larval development occurring through 3 summers (Petersen 1974, Evans and Neunzig 1996). *Nanocladius* (*P.*) sp. #5 larvae have been collected on hosts of all sizes, rarely exhibiting a correlation between host size and number of midges harbored (Pennuto et al. 2002), though this contrasts with most studies of chironomid ectosymbionts on Corydalidae (e.g., Hilsenhoff 1968, Furnish et al. 1981, de la Rosa 1992, Pennuto 1997). Obligate symbioses such as this midge/host fishfly interaction necessitate a high degree of coordination between host and symbiont because pupation of the megalopteran host occurs outside the water, leading to the death of any attached midges (e.g., de la Rosa 1992, Hayashi 1998, Jacobsen 1998). Thus, midges should complete their development prior to the host exiting the stream for pupation. To accomplish this timing, midges might be capable of determining the age of their host or they might simply use environmental cues for emergence timing. An understanding of life-history differences between hosts and symbionts is critical in determining the synchronization expected between species pairs. For example, symbionts with a life-cycle duration nearly as long as their hosts should exhibit high synchronization, whereas those with a life cycle much shorter than their host might not exhibit the same degree of coordination (Esch and Fernandez 1992).

I report the size and attachment site distribution of *N.* (*P.*) sp. #5, on its fishfly host over 3 y. Midge larvae size-frequency distributions on hosts indicated the occurrence of overlapping cohorts, so I performed laboratory rearings to determine whether emergence date of midges affected their adult masses and, thus, fecundity. Last, I performed a series of laboratory trials at different temperatures to investigate intraspecific aggression as a possible mechanism explaining the distribution patterns observed on hosts on different dates. Previous work suggested an attachment site bias and a midge density effect on emergence success (Pennuto 1998, 2000). Thus, I hypothesized that intraspecific interactions would be more intense in spring as midges migrated to the thorax for pupation.

## Methods

### *Larval dynamics in the field*

*Nigronia serricornis* larvae were collected from a 50-m riffle habitat in the Little River, Maine (Cumberland County, lat 43°41′ N, long 70°29′ W) using kicknets (0.5-mm mesh). Collections were made approximately monthly from January 1998 through February 2001. Fishflies were field-sorted from debris, placed in buckets with river water and several sticks, and returned to the laboratory where they were examined for the occurrence of *N.* (*P.*) sp. #5. This species is common in cool, stony-bottom streams of southern Maine if its host is present (Pennuto 1997, Pennuto et al. 2002). Midges attach to their host by means of a gelatinous tube, generally placed within intersegmental folds on the thorax or abdomen. The tubes are difficult to remove, remaining attached to hosts for up to 9 wk after being vacated by a midge (Pennuto 1998).

Host head-capsule width, midge number and attachment location, and (beginning in August 1999) midge head-capsule width were recorded using 10× magnification (Wild MZ-8 stereomicroscope). Each host was placed in the lid of a glass Petri dish with a small amount of river water. The bottom half of the dish was placed inside the lid, sandwiching the fishfly and reducing any movements. Midges remained within their tubes during examination. Hosts were

processed and returned to the same riffle within 8 h. Commensal prevalence (% of host population harboring ≥1 *N.* (*P.*) sp. #5), infestation intensity (mean no. of commensals per *Nigronia* examined), and commensal size-frequency distributions were calculated from these data.

Attachment site bias was assessed by comparing the observed frequency of midges on the dorsal thorax (dt), dorsal abdomen (da), ventral thorax (vt), and ventral abdomen (va) against the expectation of a random distribution on each collection date with a $\chi^2$ test. The abdomen of a fishfly is approximately twice the length of the thorax, so expected number of midges attached to both the dorsal and ventral abdomen was double the number expected on the thorax. The expected number per ventral and dorsal thorax was determined as $n/6$ where $n$ = total number of midges and 6 = the number of attachment sites (2 thoracic plus 4 abdominal). For attachment site tests, df = $(n - 1)$, where $n$ = the number of possible attachment site classes with ≥5 observations. A $\chi^2$ test also was used to compare the frequency distribution of midges on hosts to Poisson distributions on each sampling date to determine whether the number of midges per host differed from expectations. For these tests, df = $(n - 2)$, where $n$ = the number of frequency classes with ≥5 observations. Product–moment correlations were used to examine 2 relationships: 1) host size vs infestation intensity, and 2) midge size vs attachment position on each date.

*Laboratory rearings*

In September 1998, 48 fishfly hosts with attached midges were placed individually into small recirculating laboratory stream chambers as described in Pennuto (2000), and midges were monitored weekly through emergence. Laboratory stream temperatures were adjusted weekly to reflect ambient temperatures. Hosts with midges were placed in small, floating rearing chambers (described in Pennuto 2000) inside the recirculating stream chamber on the 1st day midge puparium construction was observed. After midge emergence, adults were sexed, maximum abdominal and wing width and length were recorded, and masses (ash-free dry mass, AFDM) were determined after combustion at 500°C for 30 min. The relationship between adult midge size and emergence date was examined by correlation.

*Intraspecific interaction trials*

Laboratory behavioral trials were performed in spring (6 April through 2 May) and winter (18 February through 2 March) 1999 with water temperatures at 10 and 5°C, respectively, to assess the role of intraspecific behavior in determining attachment site use by midges. These trials focused on resident midge aggression levels after an encounter with a migrating midge. Fishfly hosts harboring various numbers of ectosymbiotic midges were collected from local streams (Douglas Brook and Little River ~5 km upstream from the collection site described above) using a kicknet (0.5-mm mesh) and were maintained individually in 25-cm (ID) recirculating culture chambers. A total of 31 hosts (spring = 17, winter = 14) with head-capsule widths ≥3 mm (~2-y-old larvae) were used for behavior trials and no host was used more than once. Ectosymbiont head-capsule width was not measured, but only midges of the same approximate size were used, and no early instars were used for any trials.

A host harboring the desired number of commensals (i.e., 1, 2, or >2) was placed in a Petri dish with river water to initiate an interaction trial. A different host was retrieved and its midges removed. These midges were termed colonists. A midge was probed from its tube using a teasing needle under 10× magnification, captured in a transfer pipette, and placed on the abdominal dorsum of a new host harboring resident midges. Interactions between colonizing and resident midges were observed under 10× magnification for 30 min, recording all behaviors of the residents. Aggressive responses of resident midges were ranked as 0, 1, or 2 for increasing intensity levels (Table 1). Colonizers primarily exhibited searching and evasive behaviors upon encountering residents and, thus, were not ranked. Season and midge density effects on resident aggression levels were examined using a Scheirer–Ray–Hare 2-way ANOVA on ranked data (Sokal and Rohlf 1995). The design was unbalanced, so the exact SS for main effects and the interaction term were determined by the difference in the SS term between sequentially fitted reduced models and the full model (Analytical Software 1998).

TABLE 1. Examples of behaviors exhibited by resident *Nanocladius* (*Plecopteracoluthus*) sp. #5 during interaction trials with invaders on *Nigronia serricornis* hosts.

| Rank | Example observations |
|---|---|
| 0 | No interactions. Resident did not respond to invader touching or crawling over its tube. |
| 1 | Resident turned within its tube to face invader. Resident turned repeatedly to face both ends of its tube. Resident closed tube end. Resident extended head out of its tube and probed side to side. No contact with invader. |
| 2 | Resident extended from its tube to push or bite invader. Resident extended nearly completely (up to 90%) from its tube and flailed back and forth rapidly. |

## Results

### *General field and population observations*

A total of 947 fishfly hosts harboring 1780 midges was examined (Table 2). Midge instar size classes were estimated to be: I: ≤110 μm; II: 110–185 μm; III: 185–320 μm; and IV: ≥320 μm (Fig. 1). Midges occurred frequently on hosts on all dates, having a mean prevalence of >80% (min. = 40%, max. = 100%) and a mean infestation intensity of 1.89 larvae per *Nigronia* (min. = 0.43, max. = 3.19; Table 2). Infestation intensity and prevalence were significantly correlated ($r = 0.86$, df = 29, $p < 0.01$) and closely tracked mean host head size (Fig. 2).

Peak midge emergence occurred in May in all years as indicated by the largest number of pupae observed and the loss of 4th-instar larvae, but low levels of emergence continued throughout the summer and into early fall (Table 2). New recruits 1st entered the population in June, a month after peak adult emergence. Larvae grew rapidly to 3rd and 4th instars prior to overwintering. A large proportion of the population overwintered as 4th-instar larvae in 1999 (~75%) compared to 2000, when 4th instars were collected in late summer and early fall, but not in October (Fig. 1).

Midges exhibited a significant attachment site bias on 26 of 31 dates sampled (~84% of $\chi^2$ $p$-values < 0.05; Table 3). The 5 dates without significant attachment site bias were also the dates with the fewest number of observed ectosymbionts. Midges attached significantly more to the ventral thorax than to other sites on the host body when examining only midges occurring singly on hosts, thus omitting territorial behavior as a possible mechanism producing the observed patterns ($\chi^2 = 338.16$, df = 3, $p << 0.001$; Fig. 3). If examined simply as dorsal vs ventral, the ventral side was used most often ($\chi^2 = 10.12$, df = 1, $p < 0.005$). The frequency distribution of ectosymbionts on hosts differed from Poisson expectations only on 5 of 31 dates sampled (~16%), and there was no seasonal or sample size pattern noted in the frequency distribution data (Table 3).

There was a significant correlation between midge size and attachment position on hosts on the 14 dates with midge size observations (all $r < 0.05$). If more than a single commensal occurred on a host, the largest individual invariably occupied a thoracic position, and individuals decreased in size toward the posterior of the host (Fig. 4). Mean midge size tracked mean host size (Fig. 5), but only 7 of 31 dates (~23%) sampled had a significant correlation between host size and the number of midges harbored (Table 4). Of the 7 dates with significant correlations, none occurred in 1999 and no seasonal pattern was observed.

### *Adult characteristics*

Adult midges first emerged from the lab population on 7 May and last emerged on 17 July. A total of 38 adults were retrieved ($n = 22$ females, 16 males). There was no sex difference in the mean emergence date ($t = 1.47$, df = 36, $p > 0.05$). Prepupal plus pupal duration varied considerably (range 18 d, min. = 3 d, max. = 21 d), but did not differ between sexes (males = 11.5, females = 11.7; $t = 0.15$, df = 36, $p > 0.05$). Females were significantly heavier than males (AFDM, μg: females = 6.50, males = 3.56; $t = 4.09$, df = 36, $p < 0.001$ log-transformed data) and there was no relationship for either sex between mass and emergence date (females: $r = -0.18$, males: $r = 0.29$, both $p > 0.05$). Males were relatively thinner, but not shorter, than females and their wings were relatively more narrow, but not shorter, than females (Fig. 6). Length/width ratios for abdomens and wings were significantly smaller for females (abdomen: female = 2.23, male = 3.76; $t = 8.78$, df =

TABLE 2. Summary statistics for *Nanocladius* (*Plecopteracoluthus*) sp. #5 attached to *Nigronia serricornis* hosts in the Little River, Maine. Prevalence = % of hosts examined harboring ≥1 midge. Infestation intensity = mean number of midges per *Nigronia* examined.

| Date | Host *n* | Midge *n* | Pupae *n* | Empty pupal chambers | Prevalence | Infestation intensity |
|---|---|---|---|---|---|---|
| Jan 1998 | 34 | 63 | 0 | 0 | 94.1 | 1.85 |
| Mar | 35 | 66 | 0 | 0 | 82.9 | 1.89 |
| Apr | 25 | 43 | 0 | 0 | 72.0 | 1.72 |
| May | 46 | 25 | 6 | 13 | 45.7 | 0.54 |
| Jun | 18 | 27 | 3 | 1 | 72.2 | 1.50 |
| Jul | 28 | 51 | 1 | 3 | 89.3 | 1.82 |
| Aug | 42 | 96 | 2 | 5 | 85.7 | 2.29 |
| Sep | 32 | 99 | 0 | 0 | 93.8 | 3.09 |
| Oct | 30 | 97 | 0 | 0 | 100.0 | 3.19 |
| Nov | 31 | 82 | 0 | 0 | 96.7 | 2.65 |
| Dec | 31 | 79 | 0 | 0 | 93.5 | 2.58 |
| Jan 1999 | 30 | 94 | 0 | 0 | 96.6 | 3.13 |
| Mar | 29 | 84 | 0 | 0 | 93.0 | 2.89 |
| Apr | 23 | 50 | 0 | 0 | 95.7 | 2.17 |
| May | 38 | 52 | 13 | 7 | 78.9 | 1.37 |
| Jun | 18 | 16 | 0 | 0 | 66.7 | 0.89 |
| Jul | 34 | 67 | 1 | 2 | 88.2 | 1.97 |
| Aug | 26 | 26 | 3 | 2 | 65.4 | 1.00 |
| Sep | 27 | 46 | 0 | 0 | 81.5 | 1.70 |
| Oct | 30 | 58 | 0 | 0 | 90.0 | 1.93 |
| Nov | 28 | 63 | 0 | 0 | 89.3 | 2.25 |
| Jan 2000 | 35 | 75 | 0 | 0 | 88.6 | 2.14 |
| Feb | 18 | 18 | 0 | 0 | 61.1 | 1.00 |
| Mar | 32 | 41 | 0 | 0 | 93.8 | 1.28 |
| May | 34 | 26 | 5 | 8 | 52.9 | 0.77 |
| Jun | 35 | 15 | 1 | 0 | 40.0 | 0.43 |
| Jul | 31 | 70 | 0 | 4 | 93.5 | 2.25 |
| Aug | 44 | 68 | 1 | 0 | 81.8 | 1.55 |
| Sep | 26 | 51 | 0 | 0 | 88.5 | 1.96 |
| Oct | 26 | 64 | 0 | 0 | 92.3 | 2.45 |
| Feb 2001 | 31 | 68 | 0 | 0 | 90.3 | 2.19 |
| Total | 947 | 1780 | 36 | 45 | 82.4 | 1.89 |

36, $p < 0.001$; wings: female = 2.94, male = 3.71; $t = 6.45$, df = 36, $p < 0.001$).

*Season and density effects on intraspecific interactions*

Resident midge responses to invaders ranged from no response to nearly complete extension from their tube followed by side-to-side thrashing movements (Table 1). Biting and pushing behaviors by resident midges were observed. The intensity of aggressive behavior was significantly higher in spring with water temperatures at 10°C compared to winter when water temperature was 5°C (Table 5, Fig. 7). Commensal density did not affect aggression intensity significantly (Table 5), but aggression intensity in spring was ~2× the intensity level observed in winter when more than a single resident midge occurred on a host (Fig. 7).

## Discussion

*Population characterization*

*Nanocladius* (*P.*) sp. #5 occurred attached to its host in all months of the year with 3rd- and 4th-instar larvae most common. The observed pat-

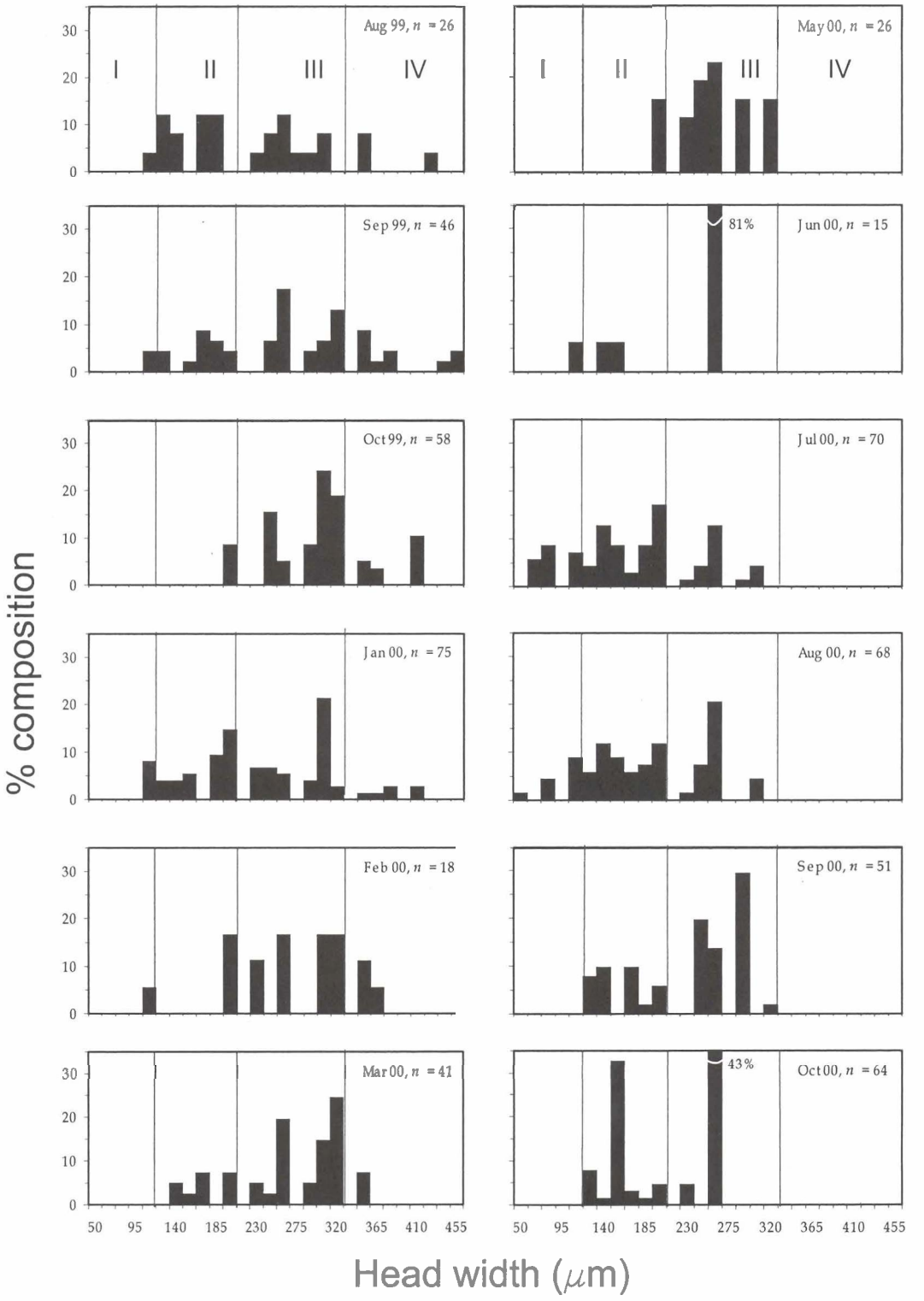


FIG. 1. Instar size (head width) categories (I–IV) of *Nanocladius* (*Plecopteracoluthus*) sp. #5 on *Nigronia serricornis* hosts in the Little River, Maine.

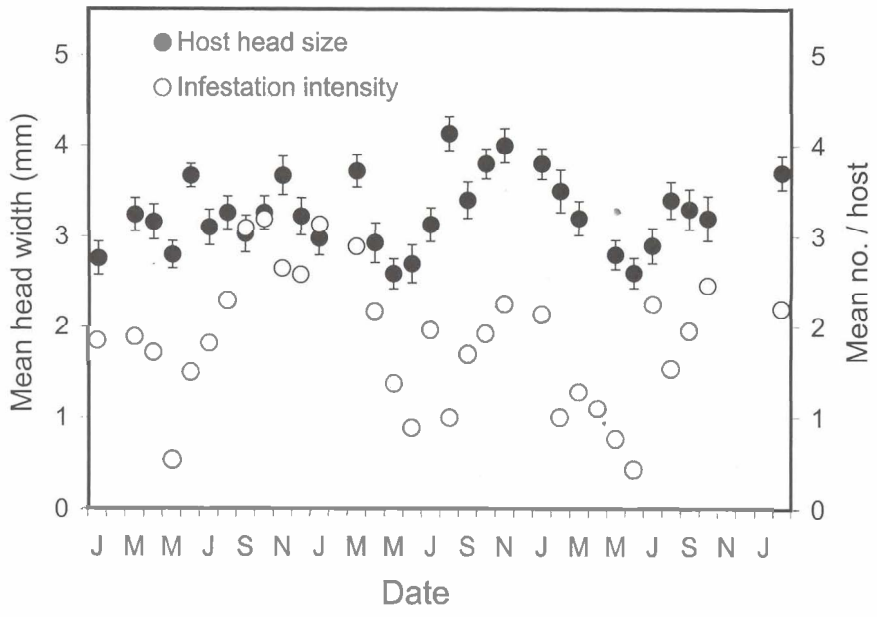


FIG. 2. Relationship between *Nanocladius* (*Plecopteracoluthus*) sp. #5 infestation intensity and *Nigronia serricornis* head width on each sampling date, beginning January 1998. Error bars = ±1 SE.

tern best reflects a univoltine, slow-seasonal life cycle (sensu Wallace and Anderson 1996) with overlapping cohorts from a prolonged emergence. The large size-class range within instars supports this observation. Jacobsen (1998) also described a complex life cycle for the parasitic midge *N.* (*P.*) sp. #1 from Pennsylvania that included 2 emergence periods and cohort splitting, and Bottorff and Knight (1987) found a similar prolonged emergence pattern for *N.* (*P.*) *downesi* in Michigan. Alternatively, *N.* (*P.*) sp. #5 in my study may have a merovoltine life history, requiring 2 y for completion. In 1999, ~50% of the observed midges overwintered as 4th-instar larvae, whereas no 4th instars were observed in October 2000 (Fig. 1). Further tracking of midge population size classes is planned to reveal their precise life cycle.

Midge population dynamics were strongly related to stream temperature. Midges developed rapidly as water temperatures began to rise in the spring and occurred more frequently on the dorsal thorax of hosts as pupation commenced. Emergence peaked in May when stream temperature was rapidly increasing and >10°C, but some emergence occurred throughout the summer and into early autumn resulting in a prolonged egg-hatching period. The 1st recruits to the next generation entered the population in June, which coincided with stream temperature maximum. Presumably, these 1st-instar larvae were progeny of emergence occurring in May. Midges grew rapidly to 3rd or 4th instars prior to overwintering, a pattern observed in commensal *Epoicocladius flavens* on *Ephemera* mayfly hosts in Europe (Tokeshi 1986). There was some winter recruitment in January and February 2000, possibly a result of early autumn oviposition because there were no pupal chambers observed during late autumn/early winter. Overwintering in both years was dominated by 3rd and 4th instars.

Larvae showed an attachment site bias on their hosts, occurring more often on the ventral thorax than any other attachment position. This same pattern was observed when assessing the attachment position of all midge larvae or when examining only those that occurred singly on the host. These observations suggest that factors other than intraspecific competition are important in determining attachment site selection by these midges, at least for midges occurring singly on hosts. Attachment site bias by ectosymbiotic midges has been observed for several other symbioses (Gotceitas and Mackay 1980, de la Rosa 1992, Giberson et al. 1996, Dosdall and

TABLE 3. Results of $\chi^2$ tests determining whether the density distribution of *Nanocladius* (*Plecopteracoluthus*) sp. #5 on their *Nigronia serricornis* host differed from Poisson expectations and whether the midges exhibited an attachment site bias on their hosts on each sampling date. n.s. = not significant.

| | Density distribution | | | Attachment site bias | | |
|---|---|---|---|---|---|---|
| Date | df | $\chi^2$ | $p$ | df | $\chi^2$ | $p$ |
| Jan 1998 | 1 | 10.74 | <0.005 | 3 | 27.71 | <0.001 |
| Mar | 2 | 2.88 | n.s. | 3 | 22.18 | <0.001 |
| Apr | 2 | 4.08 | n.s. | 3 | 31.86 | <0.001 |
| May | 1 | 0.05 | n.s. | 3 | 27.92 | <0.001 |
| Jun | 2 | 1.02 | n.s. | 3 | 18.00 | <0.001 |
| Jul | 1 | 5.11 | <0.025 | 3 | 18.88 | <0.001 |
| Aug | 3 | 1.83 | n.s. | 3 | 51.09 | <0.001 |
| Sep | 2 | 3.86 | n.s. | 3 | 58.64 | <0.001 |
| Oct | 2 | 0.74 | n.s. | 3 | 55.81 | <0.001 |
| Nov | 3 | 3.25 | n.s. | 3 | 35.80 | <0.001 |
| Dec | 2 | 4.19 | n.s. | 3 | 54.71 | <0.001 |
| Jan 1999 | 2 | 2.68 | n.s. | 3 | 38.99 | <0.001 |
| Mar | 2 | 2.41 | n.s. | 3 | 26.89 | <0.001 |
| Apr | 1 | 4.38 | <0.05 | 3 | 20.68 | <0.001 |
| May | 2 | 1.66 | n.s. | 3 | 24.09 | <0.001 |
| Jun | 1 | 0.57 | n.s. | 1 | 0.50 | n.s. |
| Jul 1999 | 2 | 4.40 | n.s. | 3 | 17.04 | <0.001 |
| Aug | 1 | 0.06 | n.s. | 1 | 3.26 | n.s. |
| Sep | 2 | 0.99 | n.s. | 3 | 31.41 | <0.001 |
| Oct | 1 | 5.39 | <0.025 | 2 | 36.91 | <0.001 |
| Nov | 1 | 2.57 | n.s. | 3 | 28.48 | <0.001 |
| Jan 2000 | 3 | 2.17 | n.s. | 3 | 53.16 | <0.001 |
| Feb | 1 | 0.88 | n.s. | 1 | 2.25 | n.s. |
| Mar | 1 | 15.36 | <0.001 | 2 | 37.54 | <0.001 |
| May | 1 | 0.98 | n.s. | 2 | 0.08 | n.s. |
| Jun | 1 | 0.17 | n.s. | 2 | 1.80 | n.s. |
| Jul | 2 | 1.52 | n.s. | 3 | 33.03 | <0.001 |
| | 2 | 1.28 | n.s. | 2 | 17.85 | <0.001 |
| Sep | 1 | 3.27 | n.s. | 2 | 26.16 | <0.001 |
| Oct | 2 | 0.76 | n.s. | 3 | 22.20 | <0.001 |
| Feb 2001 | 2 | 5.38 | n.s. | 3 | 40.31 | <0.001 |

Parker 1998, Hayashi 1998, Jacobsen 1999, Pennuto et al. 2002). Pennuto (2000) suggested the ventral thorax position might provide easy access to the dorsal thorax for pupation, and Pennuto et al. (2002) postulated that the ventral position provided both foraging and respiratory advantages. Foraging posture and behavior of hosts might direct currents under the ventral thorax, providing both food and oxygen influx to midges attached in that position.

There were few dates in my study with a significant correlation between host size and number of symbionts harbored, in contrast to several reports on ectosymbioses between midges and their hosts (Gotceitas and Mackay 1980, Tokeshi 1986, de la Rosa 1992, Giberson et al. 1996, Pennuto 1997, Jacobsen 1998). There was neither a seasonal pattern in those dates when a significant correlation was detected, nor was there any deviation between observed and expected midge frequency distribution on hosts. These observations indicate that midges locate hosts in a random manner. However, midge infestation intensity closely tracked host size, suggesting some synchrony between ectosymbiont and host life cycles. Tokeshi (1986) and Jacobsen (1998) observed a similar correlation between infestation intensity and host size. Larger hosts might be expected to harbor more symbionts because they have greater surface area for tube attachment and they are older, increasing the time available for colonization. However, if large

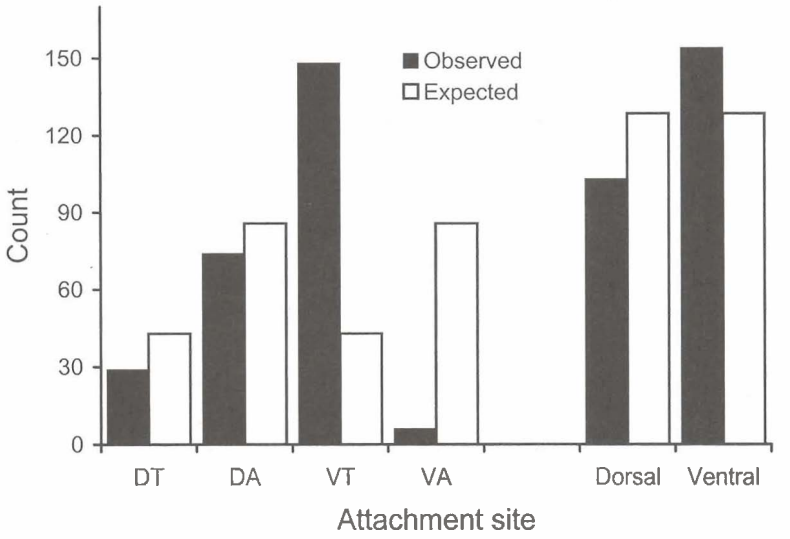


FIG. 3. Attachment site use of *Nanocladius* (*Plecopteracoluthus*) sp. #5 occurring singly on their *Nigronia serricornis* host. Data are number of midges attached to each body quadrant. DT, DA, VT, and VA refer to dorsal thorax, dorsal abdomen, ventral thorax, and ventral abdomen, respectively. See Methods for determination of expected number per quadrant.

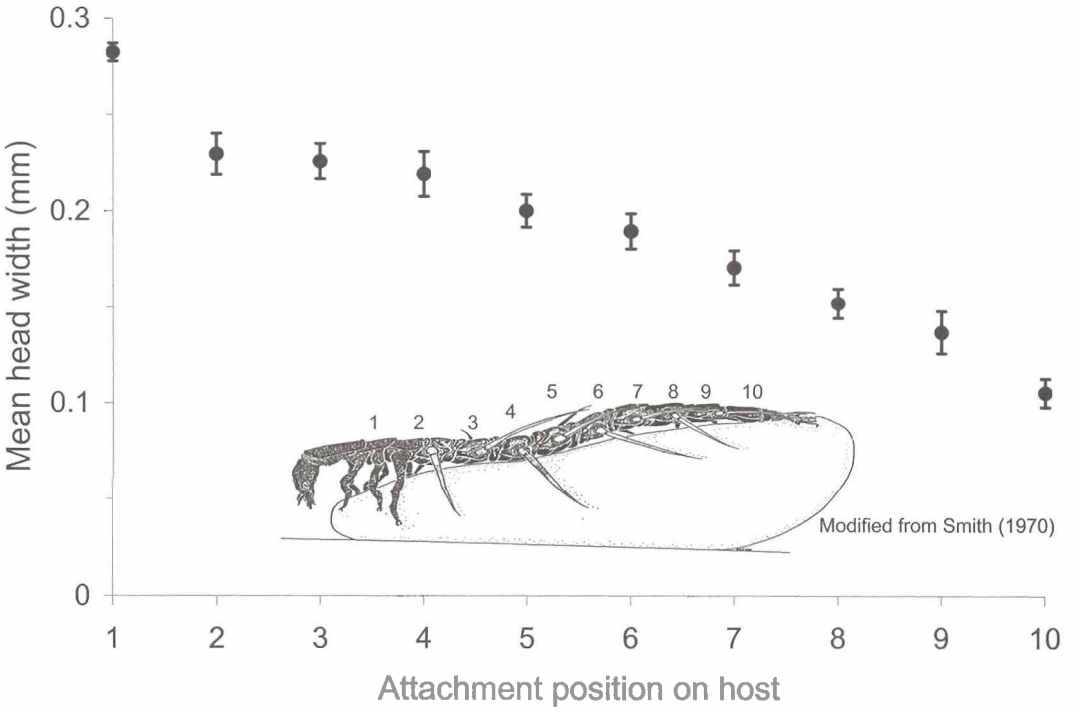


FIG. 4. Relationship between *Nanocladius* (*Plecopteracoluthus*) sp. #5 head width and attachment position on the *Nigronia serricornis* host, ranked from anterior to posterior. Error bars = ±1 SE.

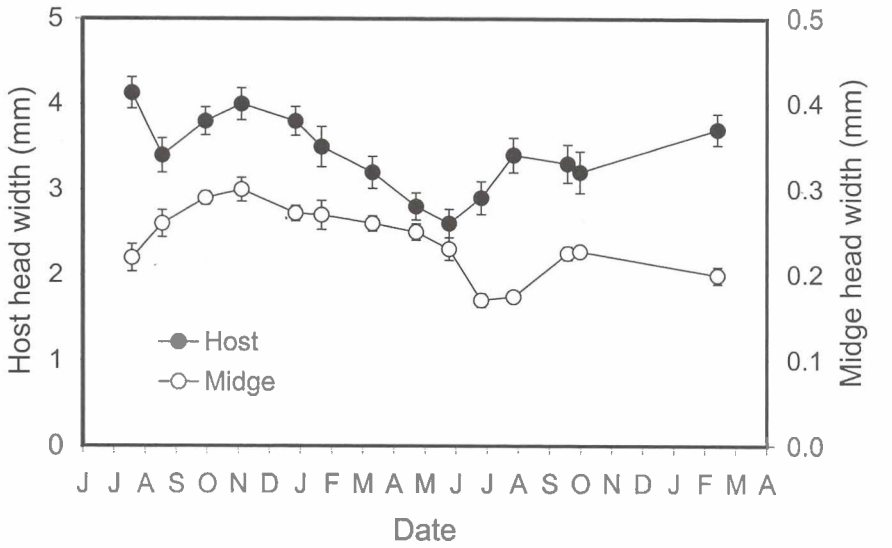


FIG. 5. Mean *Nigronia serricornis* size and mean size of *Nanocladius* (*Plecopteracoluthus*) sp. #5 determined from head capsule widths on each sampling date. Midges were not measured prior to August 1999. Error bars = ±1 SE.

hosts are more likely to emerge than small hosts, ectosymbionts should avoid them. Indeed, the largest hosts (head width >4.5 mm) in the study stream were often devoid of midges. Thus, if ectosymbiotic midges somehow avoid the largest hosts, a positive correlation between host size and number of midges would not be apparent.

Alternatively, midges attached to large hosts need to complete their life cycle prior to their host and may require a mechanism for synchronization. No studies have yet identified the cues used by midge ectosymbionts to synchronize their life cycles with that of their hosts. However, using a series of transplant experiments with various midges and their hosts, Jacobsen

TABLE 4. Correlations between *Nigronia serricornis* size (head width) and number of attached *Nanocladius* (*Plecopteracoluthus*) sp. #5 on each sampling date. The df = ($n$ − 2), where $n$ = the number of *N. serricornis* larvae observed. n.s. = not significant.

| Date | df | $r$ | $p$ | Date | df | $r$ | $p$ |
|---|---|---|---|---|---|---|---|
| Jan 1998 | 34 | 0.459 | <0.01 | Jul | 32 | 0.188 | n.s. |
| Mar | 33 | 0.011 | n.s. | Aug | 24 | 0.046 | n.s. |
| Apr | 23 | 0.495 | <0.05 | Sep | 25 | 0.160 | n.s. |
| May | 44 | 0.218 | n.s. | Oct | 28 | −0.086 | n.s. |
| Jun | 16 | −0.133 | n.s. | Nov | 26 | 0.274 | n.s. |
| Jul | 26 | 0.372 | n.s. | Jan 2000 | 33 | −0.228 | n.s. |
| Aug | 40 | −0.045 | n.s. | Feb | 16 | 0.139 | n.s. |
| Sep | 30 | −0.171 | n.s. | Mar | 30 | 0.041 | n.s. |
| Oct | 28 | 0.247 | n.s. | May | 32 | 0.461 | <0.01 |
| Nov | 29 | 0.470 | <0.01 | Jun | 33 | 0.012 | n.s. |
| Dec | 29 | 0.386 | <0.05 | Jul | 29 | 0.467 | <0.01 |
| Jan 1999 | 28 | −0.008 | n.s. | Aug | 42 | 0.064 | n.s. |
| mar | 27 | 0.128 | n.s. | Sep | 24 | 0.416 | <0.05 |
| Apr | 21 | 0.317 | n.s. | Oct | 24 | −0.213 | n.s. |
| May | 36 | 0.276 | n.s. | Feb 2001 | 29 | 0.242 | n.s. |
| Jun | 16 | −0.279 | n.s. | | | | |

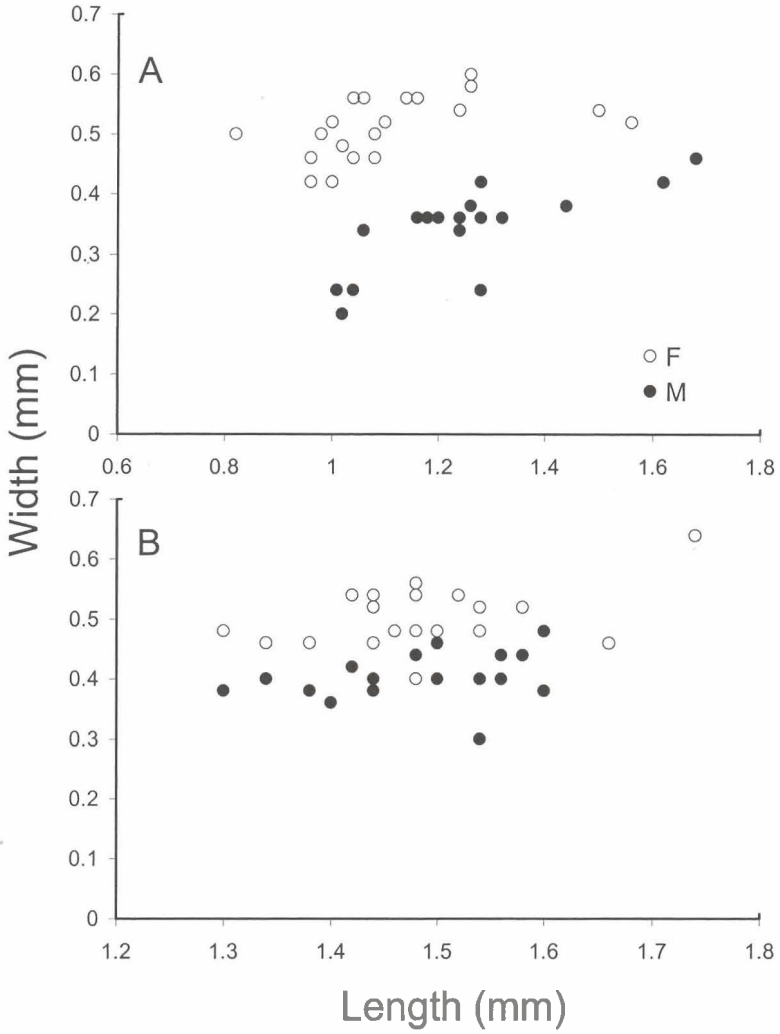


FIG. 6. Relationship between abdomen (A) and wing (B) sizes of adult male and female laboratory-reared *Nanocladius* (*Plecopteracoluthus*) sp. #5.

TABLE 5. Results of Scheirer–Ray–Hare nonparametric 2-way ANOVA examining season and density effects on aggression levels displayed by resident *Nanocladius* (*Plecopteracoluthus*) sp. #5 when encountering invading conspecifics.

| Source of variation | df | SS | $\chi^2$ | $p$ |
|---|---|---|---|---|
| Density | 2 | 253.42 | 3.46 | 0.177 |
| Season | 1 | 411.43 | 5.62 | 0.018 |
| Density × Season | 2 | 179.82 | 2.46 | 0.293 |
| Residual | 25 | 1351.38 | | |
| Total | 30 | 2196.05 | | |

(1998) showed synchronization occurred when parasitic *Epoicocladius* sp. #3 was able to arrest or enhance its development to match its host. Svensson (1976) suggested that hemolymph titers of ecdysone hormone might be accurate cues for parasitic species to synchronize with their hosts. It also is possible that there has simply been strong selection for early symbiont emergence relative to host emergence using ≥1 abiotic cues. Further studies are needed on population synchronization between ectosymbionts and their hosts to reveal the patterns within the Chironomidae.

Female-biased adult size was similar to other

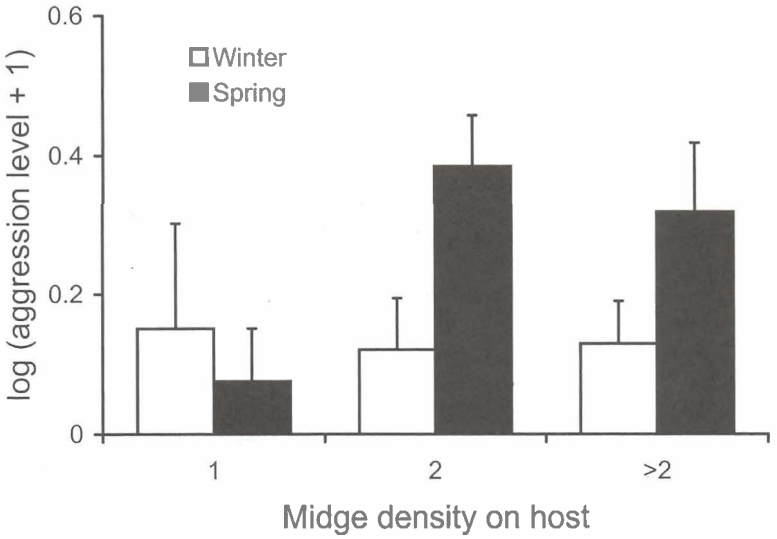


FIG. 7. Seasonal aggression intensity exhibited by resident *Nanocladius* (*Plecopteracoluthus*) sp. #5 upon encountering a conspecific colonist under different midge density conditions. Error bars = +1 SE.

Chironomidae, though adults in my study were larger than those observed by Hayashi (1998) for commensal *N.* (*P.*) *asiaticus* attached to host fishflies in Japan. No differences were observed in emergence timing of the sexes, indicating some synchronization within the population for reproduction. In addition, no relationship was found between size and emergence date for either sex. Assuming adult body size is indicative of egg production (e.g., Sweeney 1984 and references therein), emergence date does not affect fecundity in this species. However, emergence timing might have implications for likelihood of encountering a potential mate or the conditions to be experienced by hatching larvae. The latest emerging adults in the laboratory were all males (though there was no statistical differences in *mean* emergence date between sexes). Those males emerging at the late end of the emergence period would not encounter females for breeding, resulting in 0 fitness.

*Post-attachment dynamics*

Intraspecific interactions appear to be important in midge population dynamics following host attachment. Aggressive behaviors displayed by resident midges were higher at water temperature indicative of spring emergence compared to winter. These midges migrate to the dorsal thorax for pupation when water temperatures begin to increase in the spring, so they potentially encounter conspecifics enroute to pupation sites and the probability of encountering a conspecific should increase with increasing midge density on hosts. Pennuto (2000) showed that high midge density on a host (e.g., >2 ectosymbionts) resulted in low emergence success for this midge species. The coincident increase in the intensity of intraspecific interactions might lead to displacement of migrators, reducing emergence success.

Attachment position was associated with instar on all dates with available data. When >1 midge resided on a host, the largest instar always was found more anterior, in agreement with observation made by Bottorff and Knight (1987) and Jacobsen (1998) for ectosymbionts on other hosts. In addition, if an early instar midge occurred alone on a host, it resided more often on the ventral thorax than expected by chance. Together, these data indicate that attachment position on hosts is not instar-specific, but the result of intraspecific interactions and other factors making the ventral thorax a superior attachment location.

*Nanocladius* (*P.*) sp. #5 larvae are unable to switch hosts once they reach the prepupal stage and begin constructing a puparium. In the laboratory rearings, this species spent >11 d, on average, inside the puparium. All other reports suggest ectosymbiotic midges spend as little as

2 d as pupae (Bottorf and Knight 1987, Dosdall and Parker 1998, Hayashi 1998), though these studies reflect observations on the pupal stage only. Individuals spending long times in the vulnerable prepupal/pupal condition run the risk of emergence failure because of host emergence or host molt. For example, Pennuto (1998) suggested that host molting resulted in ~10% mortality of a laboratory population of *N.* (*P.*) sp. #5. However, if symbiotic midges can assess host age or developmental state and construct puparium only on young and/or stadial hosts, a longer pupal development time would not be necessarily disadvantageous. Cuticular proteins or lipids might allow midges to estimate host suitability. Cuticular proteins or lipids can be instar-specific and exuded from the epidermis (Chihara et al. 1982, Cox and Willis 1985, Souliotis et al. 1988, Willis 1996), allowing determination of host developmental condition or age without sampling host tissue (and thus being a parasite). To my knowledge, no research has been done on the cues used by symbionts to locate or select hosts, but such endeavors promise to be fruitful.

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