Association between a commensal chironomid and its mayfly host in rivers of North Westland

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Abstract

Aspects of the life cycle of an undescribed commensal chironomid *Eukiefferiella* sp. (Orthocladiinae) were examined in rivers in the vicinity of Karamea, North Westland. The four larval instars and pupa of the midge all infected mayfly nymphs, predominantly middle-instar *Deleatidium fumosum* (Leptophlebiiidae). Rates of infection of *D. fumosum* in collections from five rivers where the chironomid was found ranged from 0.85-12.2%. First and second instar chironomid larvae lay down silk for attachment, predominantly on the host thorax. In contrast, third and fourth instar larvae occupy silken tubes attached to the sides of the host abdomen. The tubes may be bound tightly to the host's gills, preventing them from beating. Typically, a single larva or pupa was found on a host, but a few mayfly nymphs were infected by two larvae, one of which was always a first instar. Larvae appeared to be indifferent as to which side of the host their tubes were attached. Results of a laboratory moulting experiment with a related mayfly species *D. vernale*, and evidence provided by cast chironomid larval exuviae incorporated into a *Eukiefferiella* tube, suggest that during summer the duration of the fourth instar was likely to be about 2-3 weeks in North Westland rivers. Host location and the ability to either remain on a host or reattach rapidly following moulting of the host are identified as critical times in the life cycle of the midge.

Keywords: commensalism - Ephemeroptera - Leptophlebiidae - Orthocladiinae - *Eukiefferiella* - *Deleatidium* - moulting - New Zealand.

Introduction

Commensalism is an association between two species in which one benefits and the other is neither benefited nor harmed. Although widespread among non-biting midges of the dipteran family Chironomidae, the only reported example of a commensal midge with an aquatic insect host in New Zealand is that of an orthoclad *Dactylocladius commensalis* whose larvae are associated with the larvae of the net-winged midge *Neocurupira hudsoni* (Blephariceridae) (Tonnoir 1923). The chance discovery

of chironomid larvae attached to nymphs of the common mayfly *Deleatidium* (Leptophlebiidae) in the Little Wanganui and Mokihinui Rivers on the West Coast of the South Island stimulated the present study of their association.

Tokeshi (1993) listed 26 species of commensal Chironomidae worldwide and an additional 11 parasitic species that feed on their hosts, which include representatives of seven insect orders. In addition to *D. commensalis*, Tokeshi noted the presence in New Zealand of *Xenochironomus canterburyensis*, a species of Chironominae whose immature stages live between the mantle and shell valves of the large freshwater mussel *Hyridella menziesii* (Forsyth & McCallum 1978). Marine midge larvae have also been found associated with the Pacific oyster (*Crassostrea gigas*) in northern New Zealand (Dinamani 1986), and are likely to be either *Semiocladius reinga* or *S. whangaroa*.

The newly discovered species associated with *Deleatidium* belongs to the genus *Eukiefferiella* and is being described formally by Dr Ian Boothroyd (Kingett Mitchell Limited, Auckland). Tokeshi (1993) recorded three other *Eukiefferiella* species with insect and molluscan hosts in North and Central/South America and Sweden. Although none of them are associated with leptophlebiid mayflies, species of *Nanocladius* and the parasitic *Symbiocladius* are associated with leptophlebiids in Honduras, and Australia and South America, respectively (Hynes 1976; Tokeshi 1993; Gonser & Spies 1997).

In the present study large numbers of mayfly nymphs were collected from rivers in North Westland in order to gain an understanding of aspects of the life cycle of the chironomid and the nature of its association with the host. Because moulting of the mayfly host means that the chironomid has to re-attach to the newly formed cuticle, or find a new host, and because field data indicated that the final stadium can be completed between moults of the mayfly host, I also carried out a laboratory trial to determine the moulting interval of *Deleatidium* nymphs at four water temperatures.

Methods

The chironomid-host association

Large numbers of potential hosts (nymphs of the mayfly *Deleatidium*) were collected from six rivers in the vicinity of Karamea, North Westland (Table 1). The rivers flow predominantly through native forest and have highly variable discharges. The larger rivers such as the Karamea and the Mokihinui are characterised by frequent bed-disturbing large floods and mean monthly temperatures ranging from about 5 to 17°C (Mosley 1982; Duncan 1992). The rivers from which mayflies were collected were 5-30 m wide at the sampling sites and had substrata dominated by large and small cobbles, fine gravel and coarse sand. They have circum-neutral pH (6.3-7.3), moderate-low alkalinity (5-29 mg l^{-1} $CaCO_3$) and conductivity ranging from 40 to 149 μS_{25} cm^{-1}. Collecting was most intensive in June and July 2002 in the lower reaches of the Little Wanganui and Karamea Rivers where they flow onto the narrow coastal plain. Collections of chironomid-infected mayflies made in the

Mokihinui River near Seddonville, 35 km south of Karamea, in February and March 2002 by Alice Bradley and Jon Harding, respectively, were also examined. Sampling was non-quantitative and involved turning over stones and washing invertebrates into hand nets (0.2 and 0.5 mm mesh). On one occasion a drift net was set up in the Little Wanganui River for six hours to collect mayfly nymphs and chironomid and mayfly exuviae. Collections were either preserved in the field in alcohol or sorted live within a few hours of collection. In the laboratory nymphs of *Deleatidium* and any other mayflies present were scanned under a dissecting microscope for commensal chironomids. Infected individuals were put aside and percentage infection for entire samples of nymphs was calculated.

Subsequently, several observations and measurements were made on the midges and their hosts. Body lengths (front of head capsule to base of posterior proleg, to 0.1 mm) and head capsule lengths (base of mandible to posterior tip of the head capsule, to 0.03 mm) of all chironomid larvae were measured with an eyepiece micrometer to delineate instars, and the attachment of chironomid larvae, pupae and their tubes or associated silk threads were recorded. Tubes were also examined for the presence of cast chironomid exuviae and any exuvial head capsules found were measured. Mayfly nymphs were sorted into morphospecies based primarily on abdominal pigment patterns and subsequently identified using Towns & Peters (1996). Body lengths of mayflies were measured from the tip of the labrum to the base of the

cerci. All nymphs hosting chironomids were measured (as above) as well as 100 randomly chosen uninfected mayfly nymphs from benthic collections made in the Little Wanganui River in June and July 2002.

Moulting experiment

To estimate the length of the intermoult period of prospective host mayflies, and by inference the length of *Eukiefferiella* larval stadia (instar duration, see below), a moulting experiment was carried out at four water temperatures. Nymphs of *Deleatidium vernale* a mayfly of very similar size and morphology to *D. fumosum* (Towns & Peters 1996) were collected from the Cust River, a tributary of the Kaiapoi River about 15 km north of Christchurch. Nymphs were brought to the laboratory on the day a trial was to begin. Moulting trials were run in constant temperature rooms or cabinets at four temperatures: 5, 10, 15 and 20°C. Medium-instar nymphs (body length 5-6 mm) were placed in groups of ten in plastic containers (volume 200 ml), containing water from the Cust River and a single periphyton-coated stone collected from the stream to provide cover and food. Numbers of nymphs used at each temperature ranged from 100-290; i.e., 10-29 groups of ten). Water in each container was aerated, continuously. For logistic reasons trials at each temperature were not run at the same time. However, all were undertaken in October and November 2002.

The numbers of nymphs moulting in the containers were monitored daily by collecting cast exuviae. Any dead nymphs were removed. Monitoring of

each trial continued for up to a week, but except at 5°C, only data for the first three days were used to calculate moulting intervals in line with the recommendation of Willoughby & Hurley (1987). At 5°C data for six days were used because moulting was infrequent and mortality of nymphs was negligible.

Estimates of moulting interval were obtained using the approach of Willoughby & Hurley (1987). They argued that the moulting periodicity of mayflies and stoneflies collected in the field would be maintained in the laboratory for an indeterminate period (a week or so), although altered by temperature as shown experimentally by Humpesch (1981). By using a large number of nymphs that are assumed to be asynchronous with respect to moulting periodicity, moulting interval can be estimated from the relationship:

$M = nd/n_1$ where, M = the intermoult period in days; n = the number of animals at the start; d = the length of the experiment in days; n_1 = the number of animals that moult.

Values of M were calculated for successive days and standard errors were calculated from the daily values.

Results

The commensal-host association

Eukiefferiella sp. has four larval instars and a pupa, all of which attach to mayfly hosts. Both larvae and pupae were found in all five months (February, March, June, July and November) in which collections were made. Measurements of head capsule length made to the nearest 0.03 mm

enabled the four larval instars to be discriminated, but an estimate of variability could only be obtained for instars 3 and 4. Instar head lengths were: instar 1, 0.06 mm (n = 7); instar 2, 0.09 mm (n = 22); instar 3, 0.13 mm (range 0.12-0.145 mm, n = 43); instar 4, 0.23 mm (range 0.20-0.26 mm, n = 43). Instars could not be discriminated on the basis of body length. Almost all larvae found (> 99%) were associated with nymphs of *Deleatidium fumosum*, which was the most abundant mayfly at the collection sites. A few larvae were found on *D. myzobranchia*, which was also common, and tended to favour faster water than *D. fumosum*. A single *Eukiefferiella* larva was found attached to a nymph of another leptophlebiid mayfly *Neozephlebia scita*.

Twenty five of the 29 first and second instar larvae (86%) were on the thorax of the mayfly host. Commonly, they were attached near the bases of the coxae on all three segments. Attachment was by the posterior proleg hooks, which grip silken threads laid down on the mayfly integument by the larva. Second instars had laid down more silk than smaller first instars. Four first instar larvae were attached to the mid- and hind femora, the side of the head behind the eye, and the base of the telofilum (middle tail), respectively.

All third and fourth instar larvae found were attached to one side of the abdomen. They occupy an open-ended silken tube into whose walls are incorporated sand grains and fine organic particles (Figure 1). The tube is usually positioned above the mayfly's gills with strands of silk passing dorsally and ventrally onto the abdominal segments and the gills, which may be
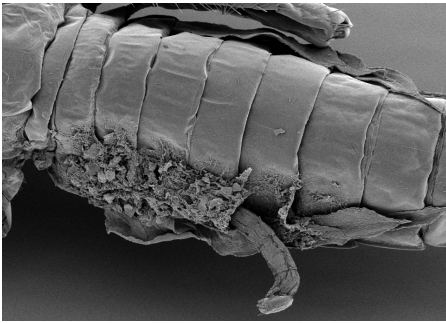
Figure 1. Scanning electron micrograph of a final instar *Eukiefferiella* larva and tube attached to the abdomen and gills of a *Deleatidium* nymph. Length of tube 1.15 mm.

bound to the abdomen so they are unable to beat (Figure 1). Shorter tubes typically occurred on abdominal segments 2 or 3, with longer ones extending from segments 1 or 2 to 4 or 5. Pupation occurs within the tube, which is closed off and may have the cast exuviae of the final instar larva incorporated into its wall. All eight pupae examined had their heads facing the posterior end of the host. Pupal tubes averaged 2.1 mm in length (SD 0.2 mm, range 1.8-2.5 mm, n = 14). One pupa was found on the side of the thorax above the bases of the legs with attachment silk extending around them onto the sterna. In a sample of 91 nymphs the number of tubes on the right (44) and left (47) sides did not differ significantly (χ^2 = 0.40, P > 0.05). However, more than twice as many tube-dwelling larvae were facing anteriorly (44) as posteriorly (19) in a scoreable sample (χ^2 = 39.7, P < 0.001).

Incidence of infection

Proportions of mayfly nymphs carrying commensal chironomids in six rivers in the vicinity of Karamea ranged from 0-12.2% (Table 1). The high infection rate in the Karamea River was found where *Deleatidium* nymphs were largely confined to cobbles scattered over a bed of predominantly coarse sand. Thus, despite their density being quite low (although not measured) and highly patchy, they were heavily infected by Chironomidae.

Typically, a single *Eukiefferiella* was found on a mayfly host, but in six instances two larvae were present. All of these included at least one first instar larva and either another first instar (twice), a second instar (once) or a third instar (three times). The third instar larvae were all on the abdomen, whereas the smaller larvae were on the thorax.

The size of *Deleatidium fumosum* nymphs used as hosts by *Eukiefferiella* was examined in collections from the Little Wanganui River in June and July 2002 (Figure 2). Size distribution of the *D. fumosum* nymphal population was very similar in the two months with the 2-3 mm length class numerically dominant. However, larger nymphs were
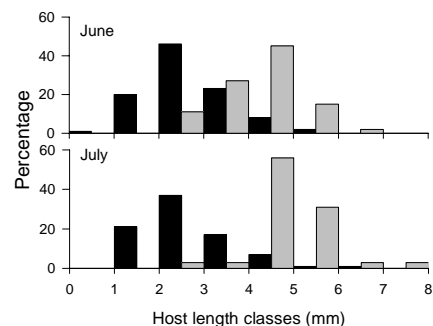

Figure 2. Relative abundance of uninfected *Deleatidium fumosum* nymphal size classes (black bars), and of nymphs infected by *Eukiefferiella* larvae and pupae (grey bars) in the Little Wanganui River, June and July 2002. Infected hosts: n = 55 (June), 29 (July). Uninfected hosts: n = a random subsample of 100 nymphs in each month.

Table 1. Rates of infection of *Deleatidium fumosum* nymphs by *Eukiefferiella* in six North Westland streams.

River	Site coordinates	Date	Larvae examined	Infection rate (%)
Karamea R	172° 08.9′E 41° 16.1′S	17.07.02	139	12.2
Little Wanganui R	172° 10.0′E 41° 21.2′S	06.06.02	1444	3.8
Little Wanganui R	172° 10.0′E 41° 21.2′S	17.07.02	1023	3.2
Little Wanganui R	172° 10.0′E 41° 21.2′S	07.11.02	31*	9.7
Blue Duck Ck	172° 06.3′E 41° 22.7′S	17.07.02	351	2.6
Oparara R	172° 08.9′E 41° 15.7′S	17.07.02	138	1.4
Elford Ck	172° 11.3′E 41° 15.7′S	07.11.02	236	0.85
Kelly Ck	172° 12.8′E 41° 15.7′S	07.11.02	434	0.0

*River in flood made collecting very difficult

selected preferentially by chironomids, which were most abundant on 4-5 mm long mayflies. There was also a weak but significant trend for longer chironomids to occur on longer mayfly nymphs ($r = 0.28$, $P < 0.05$, n = 75).

Moulting

Evidence that at least some *Eukiefferiella* larvae moult within their tubes was obtained by the finding of cast larval exuviae (including measurable head capsules) incorporated into the walls of the tubes. Thus, one final and three penultimate instar larvae had exuviae associated with them. More interestingly, a pupal tube attached to a mayfly collected in summer contained third and fourth larval instar exuviae in addition to the pupa. This indicates that the whole of the final larval stadium (intermoult period) and parts of the third instar and pupal stadia had been completed during one host intermoult period, as the tube is lost at moulting along with the old host cuticle.

Laboratory trials provided estimates of the moulting interval of *Deleatidium* larvae, and hence the likely duration of the final larval stadium of *Eukiefferiella*. *Deleatidium* nymphs moulted readily in the laboratory and, of the 680 nymphs used, 189 (27.8%) moulted. The mean estimated moulting interval increased with decreasing temperature from 7.9 days at 20°C to 32.2 days at 5°C (Figure 3). Standard errors of the mean calculated from daily estimates ranged from 4-13% at the four temperatures and were greatest at 4°C where a six rather than three day long experiment was needed to obtain sufficient data.

Average water temperature in the Little Wanganui River at the time the specimen containing multiple exuviae was collected should have been between 10 and 15°C (based on data for the
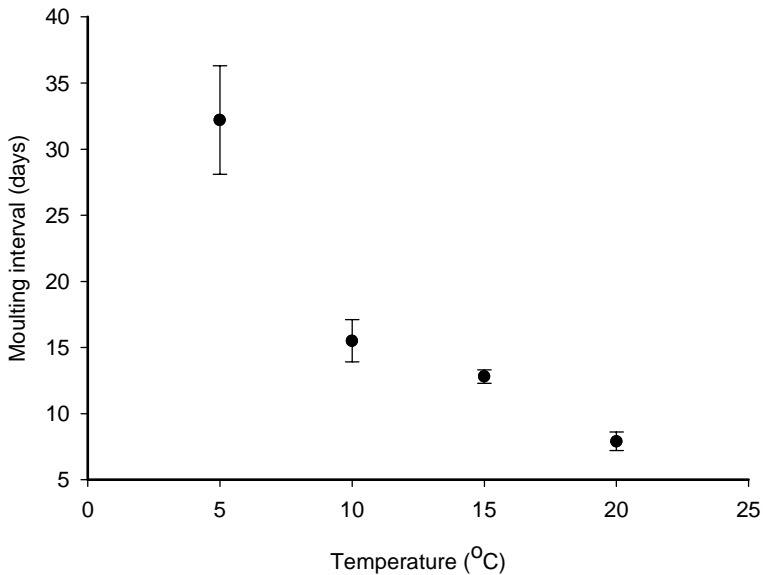
Figure 3. Moulting intervals (mean ± 1SE) of *Deleatidium vernale* nymphs at four temperatures in the laboratory, estimated by the method of Willoughby & Hurley (1987). N = 140, 100, 150 and 290 nymphs at 5, 10, 15 and 20°C, respectively.

nearby Mokihinui and Karamea Rivers; Mosley 1982), so by extrapolation from the moulting interval data for *D. vernale*, the interval between moults of medium sized *D. fumosum* is likely to have been about 12-17 days.

Discussion

Although numerous chironomid species have ectosymbiotic or parasitic relationships with other benthic aquatic insects, *Eukiefferiella* sp. is only the second such species known from New Zealand. Apart from their distinctive habitat, its life cycle stages closely resemble those of other New Zealand species of *Eukiefferiella*, and like the free-living *E. brundini* (Boothroyd & Cranston 1995), larvae and adults appear to occur year-round. Thus, several larval instars and pupae were found in all four seasons, suggesting its

life cycle is non-seasonal with numerous ill-defined cohorts. Incidence of infection of mayfly nymphs in collections in which the chironomid was found ranged from 0.85-12.2%, the highest rate being considerably lower than the maximum rates of 45-65% reported for the parasitic *Symbiocladius ephemerae* in four populations of *Ephemera danica* in the United Kingdom (Tokeshi 1995a). Dorvillé *et al.* (2000) also reported that about 31% of nymphs of a perlid stonefly *Kempnyia tijucana* in Brasil were infected by a commensal *Nanocladius* species whose life cycle could be completed on a single instar of the host.

Although *Deleatidium fumosum* was the main host of *Eukiefferiella* in the West Coast streams studied, other species of *Deleatidium* and some other genera have been found to be infected elsewhere (Ian Boothroyd, pers.

comm.). Nevertheless, *D. fumosum* appears to be an ideal host in the South Island where it is widespread and often abundant (Hitchings 2001), has a poorly synchronised life cycle, and (at least around Auckland) emergence from late-winter to autumn (Towns 1983). As a result, the middle-sized nymphs favoured as hosts by *Eukiefferiella* should be present at most times of year. A very similar combination of life history characteristics was described for *Symbiocladius renatae* and its host the leptophlebiid mayfly *Meridialaris diguilina* in southern South America (Gonser & Spies 1997). Thus, the host has two poorly synchronised and overlapping generations ensuring that larger nymphs (>4 mm long) favoured by *S. renatae* were present and infected in many months.

The physical association between *Eukiefferiella* and *Deleatidium* has little or no obvious deleterious effect on the mayfly, although some of the larger chironomid tubes bound several of the host's gills to the abdomen preventing them from beating. This could have affected the movement of well-oxygenated water over the gills, and consequently gas exchange, at least until the next moult. Why larvae move their sites of attachment from the thorax to the abdomen as they grow larger is unknown. The gut contents of five larvae from the Little Wanganui River were silt particles and diatoms, suggesting the larvae are grazers, and it is possible that the sides of the mayfly abdomen provide a more hydrologically protected environment for larger larvae, which must project from their tubes to graze, either on the body of the host, or perhaps on the substratum beneath

them. For example, Statzner & Holm (1982) demonstrated that flow velocity around the dorso-ventrally flattened nymphs of a mayfly *Ecdyonurus* declined from the thorax to the abdomen where the most extensive zone of dead water was found. Because *Deleatidium* nymphs graze on the upper surfaces of stones at night (McIntosh & Townsend 1996), they and their associated commensals will be exposed to both high and variable flow velocities.

Two interesting questions that remain regarding the life cycle of *Eukiefferiella* sp. are: how do the larvae locate their hosts, and what do they do when the host moults? Species of Orthocladiinae typically deposit strings or gelatinous masses of eggs into the water or onto submerged substrata (Forsyth 1971). The larvae that emerge from them assume a free-living existence, or may build a tube. Gonser & Spies (1997) considered that free-living first instars were the host-seeking stage of *Symbiocladius renatae*, which completes its larval and pupal development on nymphs of a leptophlebiid *Meridialaris diguilina* in Chile. Svensson (1976) found that another parasitic chironomid *Epoicocladius ephemerae* possessed "an accurate method of host finding" and speculated that a chemical stimulus was involved. Third and fourth instar *Eukiefferiella* larvae removed from their hosts in the laboratory reattached to the same or different hosts overnight (unpublished observations) indicating that host-finding ability is not confined to first instars.

When a mayfly host moults, the larval tube is lost with the detached cuticle (as shown by chironomid exuviae collected in drift nets in the

Little Wanganui River) and the larva must possess behaviour to re-attach to the moulted host, or to find a new one. It is likely that larvae sense the imminent onset of host moulting in some way and either leave the host or move onto the newly laid down exoskeleton when its predecessor splits, dorsally. In the former case the larva will need to find a new host (or reattach to the old one), whereas in the latter situation a new tube can be formed as soon as moulting is complete.

Because chironomid exuviae were not seen in most tubes it is probable that the life cycle of *Eukiefferiella* encompasses several moults of its host(s). However, cast exuviae may not always be retained in the larval tube, making this difficult to assess. Results of the *Deleatidium* moulting trial and the finding of a pupa with two successive exuvial head capsules in its tube indicate that the duration of the fourth instar is 2-3 weeks in summer at 10-15°C, and it can be expected to be longer in winter. Although the duration of different instars differs from one chironomid species to another, the last (fourth) is often the longest (Tokeshi 1995b). Therefore, it is possible that earlier instars may complete their development on a single mayfly host more frequently. A fuller understanding of the life cycle is likely to require the observation and monitoring of individuals under controlled laboratory conditions.

Acknowledgements

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