Acta Zoologica Universitatis Comenianae
46 (1): 15-21, 2004

NEW OBSERVATIONS ON PHORETIC ASSOCIATIONS OF SIMULIIDAE (DIPTERA) WITH HEPTAGENIIDAE (EPHEMEROPTERA) IN INDIA

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Abstract: During a three year investigation in fast flowing streams of the north-eastern regions of the Himalaya (India: State Himachal Pradesh, Kullu-Valley) phoretic associations of blackflies with Heptageniidae were studied. *Simulium rashidi* Lewis, 1973 and *Simulium ephemerophilum* Rubtsov, 1947 were recorded to develop on *Epeorus bispinosus* Braasch, 1980, *Electrogena eatoni* (Kimmins, 1937), *Iron martensi* Braasch, 1981, *Iron psi* (Eaton, 1883) and *Rhithrogena tianshanica* Brodsky, 1930. Further small blackfly larvae were found on a not identified species of *Rhithrogena*. Field observation proofed that at least in *S. rashidi* the attachment to a carrier is not species specific. Nevertheless, laboratory experiments revealed clear preferences for Heptageniidae, especially for *E. bispinosus*. Although larvae of phoretic blackflies proofed to be able to reattach on another individual, this seems to be done only in case the carrier died. Moreover preferences for special body regions of the Heptageniidae were evident. While larvae preferentially localised on the thorax, most of the pupae were found on the wing area of the carriers. The development of phoretics and carriers seemed to be well synchronised, because blackfly development had been finished right before the mayfly carrier emerged.

Keywords: phoretic blackflies, carrier preferences, *Simulium rashidi, Simulium ephemerophilum*, Heptageniidae

INTRODUCTION

Larvae and pupae of several blackfly species live phoretic. Their developmental stages are passed on the bodies of other arthropods. CROSSKEY (1990) mentioned 28 blackfly species developing in this specialised way. Apparently the phoretic association only has evolved in Africa and Asia and the mayfly species known to carry blackflies in Asia are closely related to the African carriers (CROSSKEY 1990). Compared to Africa, carrier diversity in Asia is quite less. There are at least three mayfly families (Heptageniidae, Oligoneuriidae, Baetidae), which are known to carry phoretic blackflies in Africa, while phoretic associations in Asia only were known between Heptageniidae and blackflies. Additionally, in Africa many phoretic associations seem to exist between blackflies and decapod crustacean. All of these Crustacean carriers belong to the genus *Potamonautes*. In Asia, no

Presented at 12. Deutschsprachiges/ 4. Europäisches Simuliidensymposium, Bratislava, 11.-13.10.2002.

associations with crabs or prawns were described so far (BURGER 1987, LEWIS 1973, CROSSKEY 1990).

During a three year investigation in fast flowing streams of the north-eastern regions of the Himalaya, phoretic associations of blackflies with Heptageniidae were studied. We tried to find out the carrier species and the phoretic species in this region. As little is known on the biology of Asian blackfly species additional laboratory studies were run to analyse larval behaviour and preferences.

MATERIAL AND METHODS

Study area

Five mountain streams (1st, 2nd and 3rd order) in the Kullu-Valley – which is between 1600 m and 2700 m a.s.l. in the catchment area of the river Beas – were examined from 1995 until 1997. The study area is about 450 km northwest of Dehli in the State of Himachal Pradesh and extends from the 31°26’ to the 31°27’ latitude and from the 76°56’ longitude to the 77°52’ longitude.

Field studies on phoretic association between blackflies and Heptageniidae

Samples of macroinvertebrates were taken by kicksampling (mesh size 350 µm) and by direct collecting from the substrate. Larvae of Heptageniidae carrying blackfly pupae or older larval stages (L6 and L7) were transferred into special cups which were placed in the stream to rear the adults. As “rearing cups” we used 250 ml PE-bottles which had 2 holes on the sides. The holes were covered with gaze, thus allowing the water pass through. Phoretic pupae and last larval stages were determined to species level according to the characters given by LEWIS (1973).

Laboratory studies

Two laboratory studies were run in order to check the preferences of phoretic larvae.

At first we tried to find out whether blackflies preferably attach to special body regions of their carriers. Mayfly larvae carrying phoretic blackflies were counted and the distribution of different development stages on wing area, thorax, abdomina, femura and heads were recorded.

In a second study we tried to figure out, whether or not phoretic *Simulium rashidi* Lewis, 1973 prefers special mayfly carriers. *S. rashidi* and its carrier, *Epeorus bispinosus* Braasch, 1980, were kept in a 10 l aquarium. Although the water was aerated, current velocity was low (< 10 cm/s). As *E. bispinosus* is not able to move gills for better oxygen supply, we supposed these individuals to die soon. Three other species, which can move their gills actively, thus are less sensitive to oxygen depletion, were also kept in this aquarium: one heptageniid species *Electrogenia eatonii* (Kimmins, 1937) and two species of the family Ephemerellidae, *Drunella* sp. Neither of the species carried phoretic blackflies when introduced to the aquarium. Blackfly behaviour was observed and recorded with an underwater lipstick video camera.

RESULTS

Field studies

We recorded five species of Heptageniidae carrying larvae and pupae of phoretic blackflies (Tab. 1): *Epeorus bispinosus, Electrogena eatoni*, *Iron martensi* Braasch, 1983, *Iron psi* (Eaton, 1883) and *Rhithrogena tianshanica* Brodsky, 1978. Further small simuliid larvae were found on a not identified species of *Rhithrogena*.

The genera *Rhithrogena* and *Iron* were already recorded from this area and also known as carriers, whereas the genera *Electrogena* and *Epeorus* were first time recorded from this area and not known as carriers.

Although a total of four species in the genus *Iron* (*I. martensi, I. nigripilous* Sintshenkova, 1976, *I. psi*, and *I. sinespinosus* Braasch, 1978) were present in our study area, phoretic blackflies were associated with *I. martensi* and *I. psi* only.

Two phoretic blackfly species were recorded (Tab. 1): The most common phoretic species was *Simulium rashidi.* It was associated with *Epeorus bispinosus, Electrogena eatoni, Iron martensi* and *I. psi. Simulium ephemerophilum,* on the other hand was quite rare. We found *S. ephemerophilum* only in one stream where it was attached to *I. martensi.*

No further phoretic blackflies were found.

S. rhithrogenophilum Konurbaev, 1984 and *S. jani* Lewis, 1973, which had been recorded previously in other parts of Asia, did not occur here (Tab. 1). Concluding from the presence of L7 and pupae, *S. ephemerophilum* and *S. rashidi* should be the only blackfly species living phoretic in this area.

None of the phoretic species was found on other substratum than on the cuticle of macroinvertebrates. As no decapod Crustacea occurred in the streams we investigated, phoretic blackflies were restricted to Heptageniidae. However, since neither eggs nor adults were found, oviposition sites and the link between egg development and phoretic life remains unknown.

I. martensi was the only mayfly species definitely carrying both the phoretic blackfly species in the field. In one stream on 2000 m a.s.l. larvae of *I. martensi* carried *S. ephemerophilum* and in another stream on 1800 m a.s.l. they were associated with *S. rashidi*.

E. bispinosus and *E. eatoni* were associated with *S. rashidi*, only. On *I. psi*, we recorded different developmental stages of phoretic blackflies. Old larvae and pupae could be determined to the species level, as *S. rashidi*. As larval colonisers were too small for species identification, we are not certain whether or not *I. psi* carries more than one phoretic blackfly and which species colonised on *Rhithrogena tianshanica*.

Phoretic blackflies did not colonise any part of the carriers' body. The body regions blackflies adhered to differed depending on the age of the phoretic. In *E. bispinosus* (Fig. 1) small and medium sized larvae of *S. rashidi* seemed to prefer the thorax region, while most of the pupae were attached to the wing area. Only single individuals were found on the abdominal area or the femora and heads. The smallest larvae of phoretic *S. rashidi* were found behind the abdominal gills. Some of the mayfly larvae carried up to three phoretic individuals, but most of them carried one larvae or pupae of blackfly only.

S. rashidi achieved highest densities in May and September on *E. bispinosus*. In May, many pupae of *S. rashidi* were found, indicating that a first generation is completed just before the monsoon rainy season (June to September) starts. During monsoon season no *E. bispinosus* carrying *S. rashidi* were found, but at the end of September, 75 % of all *E. bispinosus* caught in one stream were colonised by pupae or larval stages L6 and L7 of *S. rashidi*.

Larvae of *S. ephemerophilum* were found in high densities from June until September. Up to 50% of *I. martensi* carried phoretic *S. ephemerophilum*. Pupae occurred only at the end of September until the beginning of October.

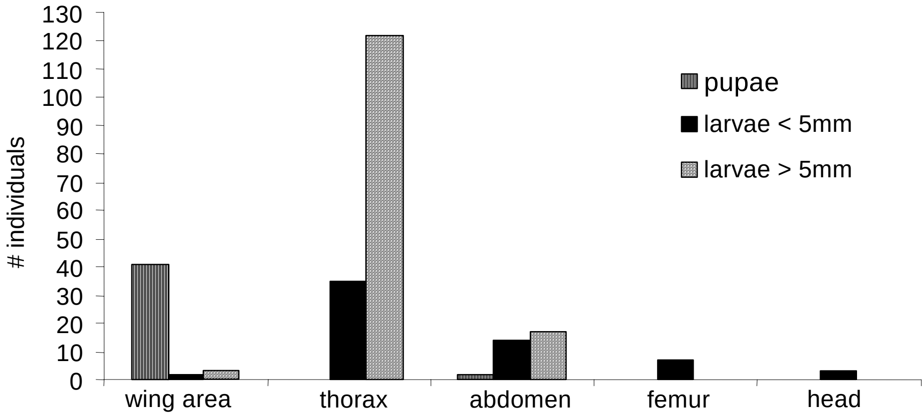

Fig. 1. The number of different sized larvae and the number of pupae of *S. rashidi* on different body regions of their mayfly carrier *E. bispinosus*.

Phoretic blackflies were only found on mayfly larvae larger than 5mm and seemed to colonise any further larval stage. However, though smaller larvae had been present at the same time, they did not carry phoretic blackflies.

At least to some extend the development of phoretic blackflies and the development of their carriers seems to be well correlated. Nearly any empty cocoon of phoretic blackflies was found on freshly emerged mayfly exuvia. Blackfly development thus seem to be finished before the mayfly emergence.

Tab. 1. Phoretic associations of blackflies and Ephemeroptera in Asia: Previous records listed in CROSSKEY (1990) and actual information on blackflies and Ephemeroptera in the Himalaya area (India).

Previous records in Asia*		Actual records in India	
Carrier	Phoretic blackfly species*	carrier	phoretic blackfly species
Rhithrogena sp.	*S. jani*[(a)], *S. rhithrogenophilum*[(b)]		
R. tianshanica	*S. alajense*[(c)] *S. ephemerophilum*[(e)]	*R. tianshanica*	Simuliidae indet.
Iron sp.	*S. rashidi*[(a)] *S. ephemerophilum*[(d)]		
Ephemeroptera indet. (genus next to *Iron*)	Simuliidae indet.[(f)]		
Ephemeroptera indet. (genus next to *Iron*)	*S. rashidi*[(a)]	*I. psi*	*S. rashidi* *S. indet.*
		I. martensi	*S. ephemerophilum* *S. rashidi*
		E. bispinosus	*S. rashidi*
		El. eatoni	*S. rashidi*

(*List of records according to CROSSKEY (1990) for (a) Pakistan, (b) Kashmir, (c) Kirgiziya and (d) USSR/Central Asia, (e) Tadzhikistan, (f) India).

Laboratory studies

In order to test the specificity of blackfly choice for the mayfly carrier, behaviour of larval *S. rashidi* were recorded in laboratory. *S. rashidi* carried by *E. bispinosus* was kept together with different mayfly species (Heptageniidae: *E. eatoni*, Ephemerellidae: *Drunella* spec.) in an aerated aquarium. The behavioural studies showed that blackfly larvae indeed may switch to another carrier. However, in our study this only happens, if the carrier died. When they were forced to leave and search for new carrier, some were watched adhering to another substratum but attached again to a new mayfly individual soon.

Though the phoretic attachment is not totally species specific, preferences were evident:

- none of the *S. rashidi* attached itself to another ephemeropteran larvae while any *E. bispinosus* were present,
- larvae of *S. rashidi* never attached to *Drunella* sp. even if no larvae of the Heptageniidae were present.

DISCUSSION

Although phoretic associations of blackflies with mayflies seem to have evolved in Asia and in Africa, at least in Asia, this phenomena is not well studied. CROSSKEY (1990) mentioned that all of the mayfly carriers currently known belong to the family Heptageniidae, i.e. the genera *Rhithrogena* and *Iron*. However, CROSSKEY (1990) also noticed that neither the range of phoresy is well documented in Asia nor are carriers known beyond generic level in most cases. *R. tianshanica* is the only one RUBTSOV (1972 – citation from CROSSKEY 1990) described in detail. In other areas *R. tianshanica* was mentioned to host *S. alajense)* [praeocc.] and *S. ephemerophilum*, and several authors reported on the genus *Iron* to be associated with phoretic *S. ephemerophilum* and *S. rashidi* by (RUBTSOV 1947, 1951, 1972 and KONURBAEV 1984 – citation from CROSSKEY 1990; LEWIS 1973).

As we regularly found phoretic association between blackflies and mayflies, our results confirm CROSSKEY'S (1990) assumption that phoresy should also exist in the Himalaya. The study also revealed that in the Himalaya further genera of the Heptageniidae serve as phoretic carriers: *E. bispinosus, El. eatoni, I. martensi, I. psi and R. tianshanica*.

For unknown reasons, the number of phoretic blackfly species in our study area was restricted: Although at least five phoretic species were described from the surrounding areas in Asia, we only found *S. rashidi* and *S. ephemerophilum*. *Simulium jani,* and *S. rhithrogenophilum* were not found.

The most common phoretic species in this investigation was *S. rashidi*. It was found attached to *I. martensi, I. psi, E. eatoni* and *E. bispinosus*. *S. rashidi* achieved highest densities, i.e. in May and in September. This is just before the start or the end of the monsoon season. In May, many pupae of *S. rashidi* indicated that a first generation is completed just before the monsoon season starts.

It seems that the next generation of *S. rashidi* only develops after the monsoon rainy season, because at the end of September, 75% of all Ephemeroptera we caught again were colonised by *S. rashidi*. However, as neither adults nor eggs were observed meanwhile, we cannot decide definitely if there were any other generation until September. *S. ephemerophilum,* on the other hand seems to develop during monsoon time.

In contrast to symbiotic associations (*Symbiocladius* sp.), the phoretic blackflies proved not to harm the ephemeropteran carrier. Comparatively measurements revealed that the body lengths of the mayfly larvae ready to emergence did not differ from individuals with or without phoretics.

Further field and laboratory observation indicate that not any phoretic associations are specific. While *S. ephemerophilum* occurs in one stream and on *I. martensi* only, at least *S. rashidi* was able to attach to several carriers.

Nevertheless, our results also indicate that the older larvae of *S. rashidi* were quite selective: they evidently preferred one species and attached to others only if there was no other choice. Moreover, preferences were restricted to one family only – i.e. species of other families (Ephemerellidae) were not taken as carriers.

Altogether, this indicates a special ability of the phoretic blackfly to sense species specific characters and probably even sense the vitality. How this is managed remains unknown. According to DISNEY (1971a, b), the number of blackflies locating on arthropod carriers is not greater than expected from random drift. This supports the assumption of CROSSKEY (1990) that drift is the key mechanism in this colonisation process. Since drift and attachment of blackfly larvae usually are passive processes (REIDELBACH & KIEL 1990), larval attachment might happen to many objects. Nevertheless, old larvae (L7) and pupae of the phoretic species never were found on other substrates in the field. As laboratory observations revealed that phoretic larvae do have clear preferences even within the family Heptageniidae, some mechanism has to be assumed. They should help larvae to decide were to leave or were to stay. CROSSKEY (1990) argues that blackflies should be able to sense hormonal changes of their carriers. If not, they should be endangered to drift off, especially in case the carrier is ready to moult.

It sounds reasonable that blackfly larvae might have the ability to sense some chemical or physiological characters of their carriers, thus leading them to their preferred species and helping to prevent accidental drift. On the other hand, we wonder whether or not these substances should be washed away by the current immediately.

Though hypothetical as well, we dare to argue that other mechanisms may also exist. Especially the sensitivity of blackfly larvae to special hydrological situations and the characters of larval silk, which causes an affinity to special substrate surface properties, could also explain the preferences and the development of phoretic blackflies. For example: even if larvae would not sense the hormonal situation, temperature could also be the factor that synchronises the developments of the phoretics and the carriers. As the behaviour of the Heptageniidae will change prior to moulting, this might cause differences in surface properties (i.e. the properties of the old skin) and will also cause hydrological disturbances. As blackflies are known to react on differences in the hydrological situation (LACOURSIÈRE 1992) as well as on changes in surface properties (KIEL 1996), phoretics thus should be able to react. Either they should drift off or they may be able to stay attached to the same (moulting) larva by switching from the old skin to the freshly emerging larva. Anyway, if we assume species specific differences of the adhesive properties (good adhesion to the body of the Heptageniidae, worse adhesion to the body of *Drunella* sp., for example) this also would explain carrier preferences.

ACKNOWLEDGEMENTS

We would like to thank Dr Arne Panesar, with whom the frame project was started and run since 1995.

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