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THE BEHAVIOR OF **ARRENURUS** LARVAE (ACARI: HYDRACHNIDEA) PARASITIZING **DIPTERA**

**SUMMARY:** In order to parasitize an adult fly, larval water mites must be pulled through the surface film of the water by the fly as it emerges from the pupal skin. The arousal response of *Arrenurus rotundus* Marshall on pupae of two ceratopogonid flies, *Palpornyia slossonae* (Coquillett) and *Sphaerornias longipennis* (Loew.) is variable and contagious so that the probability of arousing in time to grasp the ecdysing fly increases with number of mites on a pupa. In contrast, interference among larvae in large groups reduces the chances of larvae transferring from the pupa to a fly. Many larvae fail because these opposing density dependent responses reduce the number of mites per fly to a level that does not kill hosts prematurely. *Arrenurus* larvae attacking Odonata show a very different set of traits because they do not have to penetrate the surface film of the water, and have minutes, rather than seconds, to selectively attach to hosts that can support hundreds, rather than tens of larval mites.

**INTRODUCTION**

Most water mite life cycles begin with a larva that is parasitic on an adult insect. The way larvae locate a potential host, transfer to the emerging adult insect, select a feeding site, and then return to water has attracted some attention (Smith, 1988). Most aquatic water mite larvae parasitize flies (Smith & Oliver, 1986) and the number and locations of larval mites attached to adult insects are often recorded (Booth & Learner, 1978; Davies, 1959; Efford, 1963; Smith & McIver, 1984a, 1984b; Stechmann, 1978). The number of larvae transferring from pupae to the...
adult mosquitoes were recorded by Smith & McIver (1984b) but the behavior of larvae transferring from the pupa to the fly has not been analyzed. The difficulties in finding ecdysing flies and observing the larvae as they transfer during the few seconds it takes a fly to emerge from the pupa explains why so little is known about the process of fly exploitation. It is easier to obtain detailed information on how larvae transfer to Odonata because it takes a few minutes for odonate naiads to crawl from the water, seek out a perch, assume the emergence pose, and it takes several minutes to complete ecdysis. The process of transfer can be followed and analyses of transfer data have been used to explain the site preferences (Mitchell, 1968; 1969). Only a few species of the nominate subgenus of Arrenurus parasitize Odonata, thus there is a great deal of information about larvae on hosts attacked by few species but almost no information about how the hosts used by most water mites are attacked by the larvae.

Ecdysing ceratopogonid flies are easier to observe than most flies; they pupate in algal mats and ecdyse in about three days, usually within 30 minutes of sunset. Limited observations can be made during the 10 seconds of ecdysis but counts of water mite larvae transferring to adult ceratopogonids give information that is used below in the first analysis of water mite larvae transferring to adult flies. The behavior of Arrenurus larvae attacking flies is compared to Arrenurus exploiting Odonata.

**MATERIALS AND METHODS**

*Arrenurus rotundus* Marshall parasitizes two species of ceratopogonid flies, *Palpomyia slossonae* (Coquillett) and *Sphaeromias longipennis* (Loew.) (Mitchell, 1964). The fly larvae feed in floating mats of filamentous algae and pupate at the surface of the mat. Larvae of *A. rotundus* rest quietly on the pupae and remain with the pupae as they are collected from algal mats in Crane Pond (E. S. George Reserve, University of Michigan, near Pinckney, Livingston County, Michigan, U.S.A.). Pupae ecysed within about three days and ecdysis occurred just before sunset, hence, a sizable fraction of the pupae emerged during a 30 minute period.

Each pupa was collected by cutting out a section of mat in which the pupa was resting. The mat was slipped into a container, and taken to the laboratory and left undisturbed. Both the pupae and their mites remained quiescent until the flies emerged. There was no mortality among the pupae or any other response to suggest that the host or larvae were disrupted by handling.

The *A. rotundus* larvae on a pupa were counted and observed from the time the pupal skin split until the fly walked from the site of ecdysis. The mites on the pupa either attached to the newly emerged fly or were left behind on the pupal skin. These counts and the observations of activity are used to infer the behavior of water mite larvae as they transferred to adult flies.

**OBSERVATIONS**

*A. rotundus* larvae swim and crawl through mats of filamentous algae and will explore cast off pupal cases for a few seconds but if they touch a live pupa their activity slows and they crawl to the venter where they rest along the ventral membranes (Fig. 1, A). More larvae rest on the thoracic-abdominal membrane, although some larvae regularly rest on more posterior membranes. The larvae grasp the pupal cuticle but there is no evidence of feeding. If an active larva is prodded with a fine needle, it thrashes its legs rapidly but after a larva comes to rest on an abdominal segment, it does not respond to prodding unless it is pushed from the host. Even after a larva is detached, it moves slowly for a few seconds before returning to the rapid searching behavior. No larvae were seen to leave a pupa and active larvae were rarely found in the pieces of algal mat. The average number of larvae on 87 female pupae (7.5 ± 0.7) was not significantly different from the count on 78 males (6.9 ± 0.9), which justified pooling data for both sexes in the analysis of transfer.

Pupae often protrude above the surface but the strongly hydrophilic pupal cuticle pulls a film of water over the pupa. As the pupal cuticle splits for ecdysis, it opens a gap in the surface film so that the layer of air that was between the adult and the pupa is opened to the air above. The adult rises up out of the pupal case (Fig. 1, B) and the fly takes a few steps...
Fig. 1: The pupa and ecdising adult of *Sphaeromias longipennis* with *Arrenurus rotundus* larvae.

A. — Ventral aspect of the pupa with mite larvae attached to the ventral segments. B. — Fly emerging through the ecdisial split with mite larvae being pulled through the surface film by the fly. C. — *A. rotundus* larva in the water film (stippled area) drawn to the ecdisial split. The larva must attach to the wing of the host as it slides past the pupal cuticle.
within 10 seconds. The mite larvae are at rest on the pupal case when ecdysis begins. Some larvae begin to move at ecdysis and almost immediately scramble towards the ecdysial split. Single larvae differ in the time of arousal and some fail to arouse or are too late to transfer. If one larva in a group of larvae responds, the adjacent larvae are stimulated. This contagious response results in almost all larger groups becoming active within 3–4 seconds of ecdysis. Active larvae move dorsally and anteriorly to the ecdysial split (Fig. 1, B) where they push against the surface film drawn to the ecdysial split. The film of water is a barrier (Fig. 1, B–C) that larvae cannot penetrate under their own power. They may push the sharp claw-like terminal segment of the palp through the surface film. As the fly rises through the split, the mite larva grasp the host cuticle and the moment a larva grasps the emerging fly, its activity stops and the passive larva is pulled through the surface film by the host.

When one or two larvae are at the ecdysial split, they always transfer to the fly but if large numbers are at the ecdysial split, the larvae push against each other and that appears to interfere with their ability to grasp the emerging host. Some larvae in larger groups fail to transfer. After a larva is pulled out of the water by the host, it scrambles to the lateral and ventral surfaces of the thorax and venter of the fly. Most larvae attach to the ventral membranes of the anterior part of the abdomen or the thorax. There is no clear pattern or sequence of attachment and transfers occur so quickly, less than ten seconds, that the larvae cannot be traced or timed with precision. The observations suggest that two major factors affect transfer: (1) larger groups of larvae were more likely to be active in time to transfer and (2) crowding at the ecdysial split seemed to reduce the chances of a larva actually grasping the emerging host. The counts of larvae transferring can be used to test both hypotheses.

(1) Arousal variable and contagious: All single larvae transferred to the fly if they were active immediately after the pupal cuticle split. If a larva was not active by the time a fly began ecdysis, it failed to transfer. The frequency of single larvae failing to transfer is a measure of the probability of a larva arousing too late to transfer. Among the single larvae, 74% (14/19), did not respond in time to transfer. If the probability of timely arousal is similar for all larvae and one aroused larva recruits the adjacent larvae, then the chance of a group with \( n \) larvae failing to respond in time to transfer is \( n^{0.74} \). This was used to predict the number of hosts emerging with no mites and that was tested against the observed number of hosts emerging with no mites (Fig. 2, A). The chi-square test for the probability of the observed data fitting the predicted response gave a \( P > 0.975 \) \( \chi^2 (1, d.f) = 3.54 \). This supports the hypothesis that the probability of arousal in time to transfer increases with the number of larvae on a pupa.

(2) Transfer rates decline with group size: If interference among groups of larvae reduces the chances of an individual larva transferring to a fly, the portion of larvae transferring to a fly should decline with the number of larvae on a pupa. The tests for this decline excluded cases in which the larvae were not aroused. The probability of transfer (Fig. 2, B), defined as the fraction of aroused larvae transferring, declined with the number of larvae on a pupa. The significant regression \( F_{1,97} = 41.0, P > 0.999 \) supports the hypothesis, but the declining transfer rate can be explained in two ways. It could reflect either an inhibition of activity in larger groups or interference among larvae struggling to transfer. Inhibition seems unlikely because all the larvae at the ecdysial split seemed to be pushing to reach the emerging host just as vigorously as the single larvae. The curve for the data transformed to log base \( e \) follows the observed data most closely which is consistent with collisions among competing larvae rising exponentially with crowding.

The arousal and transfer curves (Fig. 2C) account for the fate of the 1193 larvae on 165 pupae. 144 larvae (12%) on 17 hosts failed to arouse and transfer to the fly and 746 (63%) larvae were aroused but did not transfer. Thus, only 25% (303) of larvae transferred to the fly and giving a load of 1.8 ± 0.2 mites/fly (Fig. 2, D). Once on the fly, the larvae seemed to attach immediately and no larvae were removed when the flies began grooming 20 seconds after ecdysis began. This gives an efficiency for transfer of only 25%. This is an underestimate of the mites ultimately succeeding because failed larvae may disperse and find another pupa.
DISCUSSION

The major puzzle posed by these data is why so many larvae failed to transfer to the host. The only other data on the efficiency of transfer, the transfer of *Arrenurus* to *Aedes* mosquitoes, also found high failure rates; from 32 to 75% of the larvae in pooled samples failed to transfer (Smith & McIver, 1984b). No density response was detected. One explanation for the low transfer rates can be found in examining the impact on the host. The load of *Arrenurus* larvae can seriously reduce both the survival and fecundity of mosquitoes (Lanciani, 1977; Smith & McIver, 1984b). Although the survival among parasitic larvae is not known, there is clear evidence of competition for space among the engorging larvae (Efford, 1966; Lanciani, 1979). If larvae are so crowded that they cannot fully engorge, the survival in the next stage, the deutonymph, may be reduced (Lanciani, 1979). As larvae in dense clusters engorge, they may press against each other with enough force to tear the host membrane to which the mites are attached. That may cause the premature death of the host and larvae. The
capacity of the host can be estimated from measurements, as was done for *A. agrionicolus* (Mitchell, 1968). The first three intersegmental membranes of the *Sphaeromias* abdomen are about 0.7 mm wide (Fig. 1, A). Larvae engorge to a width of about 0.3 mm and they rest on both sides of the membrane in the same way as they rest on the pupa (Fig. 1, A), thus, there is space for about six fully engorged larvae on each of the three anterior segments of the fly. Over 40% of the pupae carried more than six larvae before ecdysis but only 5% of the flies carried more than six larvae after transfer, thus, the observed numbers of larvae on emerging flies were below the number that could engorge on the host.

Selection could act on the traits of the mites if there is heritable variation in the arousal response. If some larvae always respond in time to transfer and recruit adjacent larvae, the responsive larvae might often die because of overloading the host. Similarly if the probabilities of transfer are higher, the larval mites might not have enough space for engorgement. If an increase in the probabilities of arousal and transfer also increased the chances of premature deaths among the hosts and their mites, then selection would be either neutral or act against the traits. There is undoubtedly yearly year to year variation in the relative densities of pupae and mite larvae. In years when few larvae are on the pupae, it would be advantageous to arouse in time to transfer but when the densities of mites per pupa are high, an efficient transfer could increase loads to a level that would increase the deaths among both hosts and mites. Conversely, low transfer rates when larvae are very abundant would increase the survival of larvae on flies by reducing the loads.

*Arrenurus* larvae attacking Odonata do not face the barrier of the surface film because water evaporates from the host leaving the larvae in air when ecdysis begins. Larvae can crawl directly from an odonate naiad to the adult. Arousal times can vary by one or two minutes and still be so closely correlated with the events of odonate ecdysis that they determine which part of the host will be encountered (Mitchell, 1961). Larvae have a few minutes to select an attachment site. The well known *Arrenurus* parasites of the damselfly, *Cercion hieroglyphicum*, (Mitchell, 1968, 1969) illustrate this; *C. hieroglyphicum* is just over 30 mm long. Ecdysis takes about 8 minutes and the adult rests in the pupal case for another 10 minutes before the cuticle hardens enough to support the body. Attachment sites appear to be determined by the time of transfer: larvae that are active early encounter the thorax, larvae that transfer late arrive on the abdomen. *Arrenurus* larvae on Odonata have 10 to 20 minutes for loading and exhibit precise loading behaviors as they attach to the host. The larvae space themselves along the membranes as they attach and, if the first site is crowded, they may move to the other side or to an adjacent segment (Mitchell, 1969). The 10 mm long ceratopogonid flies ecdyse in 10 seconds and the fly walks away in 20 seconds. The time for fly ecdysis may be so short that the larvae have no time to select attachment sites before the fly attempts to dislodge mites.

The capacities of the attachment sites also differ. The lateral membranes between the dorsal and ventral abdominal plates of *C. hieroglyphicum* extend down both sides of the abdomen to give a combined attachment area that is about 52 mm long. *Arrenurus* larvae engorge to approximately 0.3 mm, hence, this small damselfly can support about 170 larvae. Various data on the loads of mites on Diptera suggest a symmetrical loading on flies (Efford, 1963). There might be site selection on the host but the loading data for *A. rotundus* suggest an alternative explanation; symmetry could be an indirect consequence of the limitations of space and time. If the numbers of mite larvae arriving at the left and right side of the ecdysial split are not equal, the side with fewer larvae would have higher transfer rates, while a smaller portion would transfer from the side with the larger number of mites. The negative density dependent success in the struggle for a position from which to grasp the emerging ceratopogonid could balance loads without any active selection of sites by the larvae. The small flies considered by Efford (1963) may emerge in so short a time that one larva at the ecdysial split would transfer more often than two larvae that are pushing each other for a position from which to grasp the host. Competition for a position is a simple and parsimonious explanation for symmetrical loads of mite larvae on flies.

The capacity of an odonate is so great that larva-induced host mortality is rare. There can be massive
loads of hundreds of larvae which will engorge before
the host is so weakened that it dies. It has been argued
that heavily loaded hosts are most likely to die in
strenuous activities over water and the premature
death of a few heavily loaded hosts could return
hundreds of larvae to the water. That could play a
role in sustaining some populations of odonate para-
sites as demonstrated in a population in which 14% of
the hosts carried over half the parasitic larvae (Mrz-
chell, 1967). Flies cannot carry enough mites to
make that tactic possible.

These data raise the question of whether the traits
of A. rotundus represent an evolutionary equilibrium;
is the advantage for a more efficient transfer of larvae
from the pupa to the adult fly balanced by the detri-
mental effect of overloading hosts? If such a balance
occurs among fly parasites, it would mean that what
appears to be a behavioral regulation of host exploi-
tation by fly parasites is only a reflection of what
happens when transfer rates are time limited and host
capacity is so low that nothing is gained by a more
efficient transfer. That is fundamentally different
from the exploitation of odonates by water mites. The
water barrier evaporates allowing the leisurely trans-
fer of all the larvae and with ample time to select
attachment sites. In addition, a few odonate hosts
carrying several hundred larvae could play an im-
portant role in maintaining a local population (Mrz-
chell, 1967, 1975). The number of larvae on one
heavily loaded odonate equals the number of larvae
carried by over hundred flies bearing the largest pos-
sible number of larva.

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