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Site-specific effects of parasitism on water balance and lipid content of the parasitic wasp *Nasonia vitripennis* (Hymenoptera: Pteromalidae)

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Abstract. The site of parasitism on a host selected by the ectoparasitic wasp *Nasonia vitripennis* affected the water composition of developing larvae. Second instar wasp larvae developing on anteriorly-parasitized pharate adults of *Sarcophaga bullata* contained 12% more water by mass than wasps feeding on the posterior end of the fly. Similar differences, although less pronounced, were detected in third instar larvae and are attributed to a greater dry weight (fat). Regardless of the amount of fat present in wasp larvae, rates of water loss and oxygen consumption were the same. This suggests that variation in parasitoid fat content did not contribute to water conservation. Parasitism resulted in an elevation in the hemolymph and fat body lipid content of *Sarcophaga bullata*, but the induced-hyperlipaemia was most pronounced in posteriorly-parasitized flies. Wasp larvae reared on the latter type of host contained the most extractable lipid, implying that variation in parasitoid fat content simply reflects differences in host composition. Differences in the quantity of host lipids did not alter the duration of parasitoid development.

INTRODUCTION

Upon encountering a host, the ectoparasitic wasp *Nasonia vitripennis* drills through the hardened fly puparium, injects venom, and then deposits eggs (Edwards, 1954; Dawei & Dingxi, 1987). Prior to egg hatch and parasitoid feeding, fly development ceases and a series of changes in host metabolism is induced that seem to facilitate parasitoid growth (Rivers & Denlinger, 1994a). These effects are dependent not only on the host species but also on the site of envenomation. This is evident when comparing differences in the rate of host decay (Rivers & Denlinger, 1994b) and rate of water loss for anteriorly and posteriorly envenomated pupae of *Sarcophaga bullata* (Yoder et al., 1996). Fly pupae envenomated posteriorly lose water at a slower rate than anteriorly envenomated pupae (Yoder et al., 1996), and would be expected to provide a more suitable intrapuparial environment for developing wasp larvae because the deterioration of host tissue should be slowed. These site-specific responses are consistent with the wasp's oviposition preference for posterior regions of the fly host (Rivers & Denlinger, unpublished observations).

In this study, we evaluated whether the site of parasitism affects the lipid content, water relations, and rates of oxygen consumption of *N. vitripennis* by comparing larvae reared on anteriorly or posteriorly parasitized pharate adults of *S. bullata*. We also tested the hypothesis that larvae of *N. vitripennis* will contain more lipid when reared on fly hosts with elevated hemolymph lipid levels (posteriorly envenomated flies) than when developing on flies with a lower fat content (anteriorly envenomated flies).

MATERIAL AND METHODS

Parasitoid and host rearing

A laboratory colony of *N. vitripennis* (Walker) (Hymenoptera: Pteromalidae) was maintained as described (Rivers & Denlinger, 1994b). Adult wasps were reared on pupae of the flesh fly, *Sarcophaga bullata* (Diptera: Sarcophagidae), at 25°C with a daily light : dark cycle of L 15 : D 9. All female wasps used for parasitizing fly hosts were 3–7 days old after emergence from host puparia.

Colonies of *S. bullata* were maintained as described (Denlinger, 1972). To generate nondiapausing pharate adults, adults and larvae were held at 25°C in a light-dark regime of L 15 : D 9.

Host exposure to parasitoids

Puparia of *S. bullata* (5 days after pupariation) were wrapped in aluminium foil so that only the anterior or posterior third of each puparium was available to wasps. This restricted the site of parasitoid venom injection and egg deposition, allowing comparisons to be made between anterior and posterior parasitism of *S. bullata*. Each host was exposed to a single female of *N. vitripennis* for 6 h in a plastic 1 oz. cup (Dixie). After exposure, the adult wasps were discarded and the parasitized hosts were maintained at 25°C, L 15 : D 9. On each day following parasitism (7 days in all), 15 parasitized flies were used for lipid extractions.

A second group of parasitized *S. bullata* was kept separate and used to monitor the duration of each developmental stage of *N. vitripennis* from egg to adult eclosion. Progression of wasp development at 25°C was monitored every 12 h by removing the operculum of each fly puparium and determining the developmental stage of the parasitoids using the criteria of Schneiderman & Horwitz (1958). Second and third (final) instar larvae of *N. vitripennis* were collected from some of the fly puparia for studies of water balance and total body lipid extractions.

In a parallel set of experiments, the anterior or posterior end of wrapped fly puparia was removed following exposure to *N. vitripennis*, and the parasitoid's eggs removed. Envenomated *S. bullata* were kept at 25°C, L 15 : D 9 and used for oxygen consumption measurements.

Water balance

Characteristics were derived gravimetrically using an electrobalance (Perkin-Elmer AD-4; precision of ± 0.2 mg SD and accuracy of ± 6.0 mg at 1 mg). Larval weight of *N. vitripennis* was monitored in response to exposure at various relative humidities. Insects were weighed singly. Test relative humidities (% RH) were generated by saturated salt solutions (Winston & Bates, 1960) or glycerol-water mixtures (Johnson, 1940). Calcium sulfate provided 0% RH (Toolson, 1978). So that changes in weight largely reflect changes in body water, wasps were held at 93% RH without food and water for a day (Arlian & Ekstrand, 1975) followed by partial dehydration of 6–8% body mass (Wharton, 1985; Yoder et al., 1994). Specimens were transferred to the weighing pan with an aspirator and returned to humidity chambers within a minute. No anaesthesia was used, and wasp larvae were not observed to defecate, excrete, or purge their gut during the course of experiments.

For water content, insects were weighed and then dried (0% RH, 90°C) to constant weight. The amount of water lost (water mass) was expressed as a percentage of initial body weight. Rates of water loss (transpiration; integumental plus respiratory water loss) were determined at 0% RH, 22–24°C by daily weighings. The slope of a regression through a semi-log plot of the ratio of the water mass at time t (m_t) to original water mass (m_0) against time (ln m_t/m_0 vs. time) is the rate of water loss (%/h). Water gain from the air was tested at various relative humidities, 22–24°C, by daily weighings. The lowest relative humidity where mass remained constant for 5–6 days is an equilibrium humidity. It designates the relative humidity above which water can be absorbed, and is the point where water balance (water gain = loss) is estimated to be achieved under these conditions (determinations from Wharton, 1985).

Lipid extractions

Hemolymph and fat body samples from *S. bullata* parasitized either anteriorly or posteriorly by *N. vitripennis* were prepared as described (Rivers & Denlinger, 1995b). Briefly, five microliters of hemolymph and the entire fat body pellet (0.5 ml) from each host were transferred to glass test tubes containing 1 ml chloroform, and the lipids were extracted (modified from Bligh & Dyer, 1959). Lipids were then converted to sulfonic acid derivatives and quantified colorimetrically using vanillin reagent (Van Handel,

1985). Sesame oil (Sigma) in chloroform was used as the standard. Total body lipid from second and third instar wasp larvae was quantified similarly (Rivers & Denlinger, 1994a).

Oxygen consumption determination

The rate of oxygen consumption was determined for each developmental stage of *N. vitripennis* using a Scholander volumetric respirometer (modified from Denlinger et al., 1972). Preliminary trials revealed that eggs, and wasp larvae dehydrated rapidly and died 20–24 h after removal from host puparia in the experiment. Thus, we left wasps encased within host puparia during measurements. Three anteriorly-parasitized pharate adults (5 days after pupariation) of *S. bullata* were placed in each respirometer vessel, and the consumption of oxygen at 25°C was recorded until parasitoids eclosed as adults. Experiments were repeated using posteriorly-parasitized fly hosts. Oxygen consumption rates were also determined for envenomated (injected with venom, but the parasitoid's eggs were removed) pharate adults of *S. bullata*, and these values were subtracted from oxygen consumption rates recorded for *N. vitripennis* to correct for any contribution from the host.

RESULTS

Water balance characteristics of *N. vitripennis*

Second instar larvae of *N. vitripennis* that had been feeding at the posterior end of the host (choice oviposition site) contained about 12% less water by mass than larvae that had been feeding at the anterior end of *S. bullata* (66 vs. 78%) (Table 1). A difference in water content was also observed, though not as pronounced, in third (final) instar larvae (76% and 71%, anterior- vs. posterior-oviposition, respectively).

TABLE 1. Comparison of water balance characteristics of *Nasonia vitripennis* as second and third (final) instar larvae deposited at different sites (anterior vs. posterior) on pupae of the flesh fly, *Sarcophaga bullata*.

Oviposition site	Water balance characteristic of larvae of *Nasonia*					
	Wet weight (mg)	Dry weight (mg)	Water mass (mg)	Body water (%)	Water loss (%/h)	Equilibrium humidity (% RH)
Anterior						
2nd instar	0.0452±0.004[a]	0.0100±0.004[a]	0.0352±0.003[a]	77.88±0.34[a]	3.79±0.017[a]	≥ 98[a]
3rd instar	1.328±0.03[b]	0.319±0.02[b]	1.009±0.03[b]	75.98±0.49[b]	0.269±0.021[b]	≥ 98[a]
Posterior						
2nd instar	0.0447±0.003[a]	0.0153[c]±0.003[c]	0.0294±0.002[c]	65.77±0.42[c]	3.82±0.021[a]	≥ 98[a]
3rd instar	1.336±0.04[b]	0.383±0.02[d]	0.953±0.02[d]	71.33±0.43[d]	0.272±0.015[b]	≥ 98[a]

Oviposition was restricted by wrapping host puparia in foil so that either the anterior or posterior regions were available to the wasp. Wasp larvae were predried 4–6% before use, and studies were performed at 22–24°C. Values followed by the same letter within a column are not significantly different (ANOVA; $P > 0.05$). Percentage data were arcsin transformed prior to analysis (Sokal & Rohlf, 1969). (N = 3 replicates of 15 larvae each.)

Other water balance characteristics for the wasp larvae were not altered by the site of egg deposition (Table 1). Smaller second instar larvae lost water at a faster rate than third instars as a likely consequence of a larger surface area to volume ratio, and both lacked the ability to absorb water vapor. Regional oviposition affected larval dry weight only.

Lipid content of *N. vitripennis*

The levels of lipid in *N. vitripennis* were much higher in late (48 h into instar) second instar larvae than in early (24 h into instar) and late third instar larvae: both the absolute amount and the percentage body lipid were nearly 10-fold higher in late second instars than in third instar larvae, regardless of the host body region used for oviposition (Table 2). When expressed as a percentage body weight (initial), lipid accounted for 35.8% of the body weight of second instar larvae developing on posteriorly-parasitized flies and 30.2% for second instars reared on the anterior body regions of the host. By the end of the third larval stadium, differences between the two groups were not significantly different prior to pupation (Table 2).

TABLE 2. Total body lipid content of larvae of *Nasonia vitripennis* reared on anteriorly or posteriorly-parasitized pharate adults of *Sarcophaga bullata*.

Host body region	Age of wasps	Total body lipid	
		mg/g	%
Anterior	late second instar	317.1 ± 7.3a	30.2 ± 0.9a
	early third instar	35.3 ± 1.3b	3.5 ± 0.1b
	late third instar	23.1 ± 0.8c	2.5 ± 0.1c
Posterior	late second instar	399.0 ± 6.1d	35.8 ± 0.8d
	early third instar	44.2 ± 1.2e	4.4 ± 0.1e
	late third instar	23.9 ± 1.0c	2.3 ± 0.1c

Wasps termed as "early" were 24 h into the instar; "late" larvae were 48 h into the instar. Values (X ± SEM of 20 larvae from each age group) followed by the same letter within the same column do not differ significantly at $P > 0.05$ (Student-Newman-Keul's multiple comparisons tests; Sokal & Rohlf, 1969).

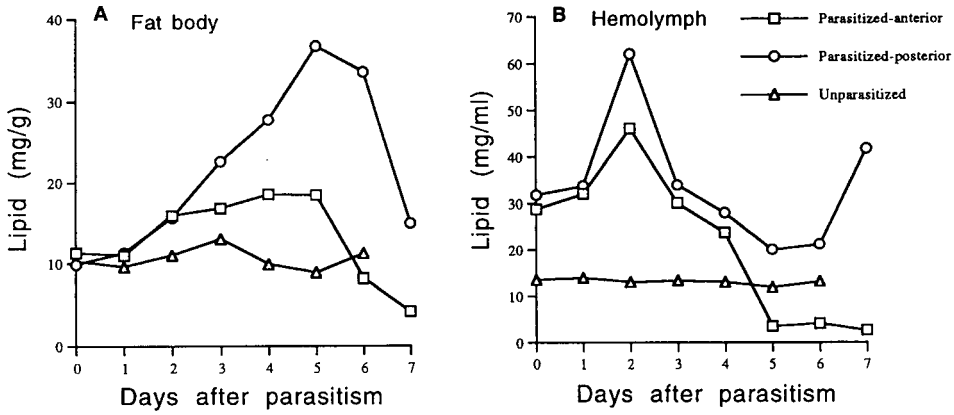

Fig. 1. Lipid composition of (A) fat body and (B) hemolymph in nondiapausing pharate adults of *Sarcophaga bullata* following parasitism in the anterior or posterior body region by *Nasonia vitripennis*. (N = 15 pharate adults at each time interval.) Hosts were maintained at 25°C, L 15 : D 9 following parasitism.

Lipid content of parasitized flies

Lipid levels in the fat body of unparasitized flies remained fairly constant throughout pharate adult development (Fig. 1A). As expected (Rivers & Denlinger, 1995b), when pharate adults of *S. bullata* were parasitized in posterior body regions by *N. vitripennis* the fat body lipid content steadily increased during the following 4 days (Fig. 1A). The peak in lipid titer was followed by a rapid decline that, by the 7th day post-parasitism, was reduced to quantities found in the fat body of unparasitized controls. Similar increases in fly lipids, although less pronounced, were observed when anteriorly-parasitized (Fig. 1A).

Consistent with the observations in fat body from unparasitized flies, hemolymph lipid levels remained relatively constant throughout pharate adult development (Fig. 1B). In flies parasitized in either the anterior or posterior body regions, hemolymph lipid levels rose nearly 3–5 times higher than observed in unparasitized flies (Fig. 1B). By 3 days post-parasitism, levels of lipid began to decline in both groups of parasitized flies and approached control titers by 5–6 days following parasitism (Fig. 1B). The lipid content continued to drop in anteriorly-parasitized pharate adults and by day 7 post-parasitism, these flies contained only 1/3 the content extracted from unparasitized flies and 1/5 the content extracted from posteriorly-parasitized flies (Fig. 1B).

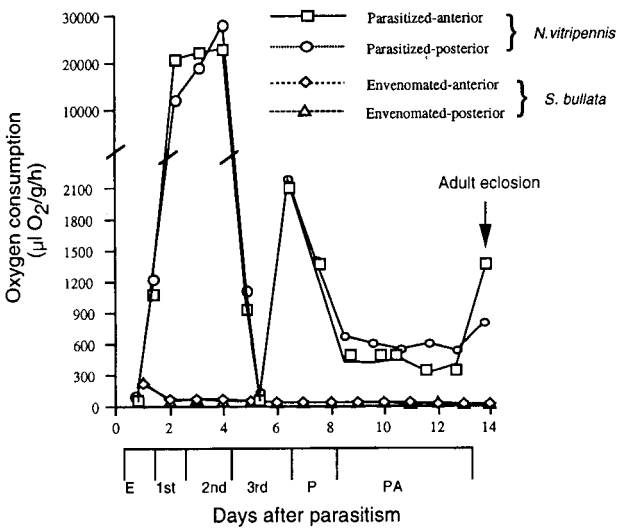

Fig. 2. Changes in the rate of oxygen consumption as development progressed from egg to adult eclosion in *Nasonia vitripennis*. The rate of oxygen consumption (µl O_2/g/h) at 25°C was determined for wasps developing on the anterior or posterior body regions of the host, *Sarcophaga bullata*. Wasps remained encased within the fly puparia throughout development, thus the rate of O_2 consumption for *S. bullata* injected with venom, but which contained no developing parasitoids (envenomated), was determined for comparison. Points represent the mean of 120 wasps and vertical error bars lie within the confines of graph symbols. PA – pharate adult.

Rate of oxygen consumption in *N. vitripennis*

The rate of oxygen consumption in *N. vitripennis* was highest in first and second instar larvae, the stages of development in which the largest changes in body weight occurred: larval weight increased from 0.003 ± 0.001 mg (X ± SEM, N = 30) for first instars to 0.040 ± 0.009 mg for second instars (Fig. 2). As development progressed, oxygen consumption dropped sharply in 48 h-old third instar larvae, and then elevated at the onset of pupation. By completion of the pupal stage, the rate of oxygen consumption declined to 1/3 of the rate observed at the onset of pupation and remained relatively constant (420–685 µl/g/h) during the 5 days of pharate adult development

(Fig. 2). The site of oviposition on the host (anterior or posterior) did not influence oxygen consumption rates in developing parasitoids until adult eclosion, where wasps reared from posteriorly-parasitized hosts had a higher rate of oxygen consumption than those reared on anteriorly-parasitized hosts.

Since the developing parasitoids were not removed from the host during measurements of O_2 consumption, it was necessary to determine what contribution, if any, the parasitized fly made to the rates calculated for *N. vitripennis*. Pharate adults of *S. bullata* injected with venom but which contained no developing parasitoids (envenomated) consumed less than 100 µl O_2 g/h throughout the 14 days of the study, regardless of the site of parasitism (Fig. 2).

Duration of wasp development on parasitized flies

At 25°C, *N. vitripennis* required 14.2 ± 0.3 (X ± SEM, N = 45) days to develop from egg to adult eclosion, regardless of the site of egg deposition on the host. The duration of each developmental stage of *N. vitripennis* was not altered when the wasps developed on hosts parasitized in either the anterior or posterior body regions.

DISCUSSION

We have reported previously that water conservation of *N. vitripennis* is enhanced by the puparium of its fly host (Yoder et al., 1994). Wasp larvae reside within the host puparium; specifically, in the space created at pupation between the fly pupa and puparium (Whiting, 1967). This environment is an unstirred air-space, and was previously thought to maintain higher humidities in the air in contact with the parasite's integument due to host water loss (Yoder et al., 1994). Wasps developing in this space but on different regions of the host may display different water relations due to site-specific effects of parasitism. In the present study, however, we found no differences in the water loss rates for parasitoid larvae that could be attributed to the fly body region used for oviposition. This is consistent with the view that nondiapausing larvae of *N. vitripennis* lack the ability to absorb water vapor and obtain all of their water from feeding on the host (Yoder et al., 1994).

Transpiration was found to decrease with increasing age of wasp larvae, but these differences were not dependent on the host body region used for envenomation. The smaller, second instar larvae of *N. vitripennis* lost water at a faster rate than third instar larvae, and this is a consequence of smaller insects possessing a large surface area in relation to their volume (Schmidt-Nielsen, 1984). It is also likely that differences in the rate of oxygen consumption contributed to the rate of water loss: second instar larvae consumed 10 times more oxygen per gram of tissue than late third instars, which is known to elevate respiratory water loss (Wharton, 1985).

Terrestrial arthropods must balance water loss by water gain (Wharton, 1985). Water gain in *N. vitripennis* is through larval feeding on host fluids and tissues. Feeding activity thus influences total body water composition. In most insects, water constitutes about 70% of the total body mass (Hadley, 1994), and this closely approximates the water content of second and third instar larvae. The quantity of body water was influenced by the site of parasitism: larvae that developed on posteriorly-parasitized hosts contained less water than those reared on anteriorly-parasitized hosts. Variation in body water content is attributable to fat (Hadley, 1994); the less water is present, the more fat (dry mass). Indeed, more fat

was extracted from wasp larvae reared on posteriorly-parasitized flies than from those developing on anteriorly-parasitized hosts.

Generally, a reduction in the water content of an insect leads to water conservation and low water loss rates (Wharton, 1985). We have shown, however, that the rate of water loss in larvae of *N. vitripennis* is the same despite variation in fat content. This suggests that differences in the quantity of fat did not contribute to the conservation of body water.

When comparing different species of flies used as host by *N. vitripennis*, elevation of host lipids appears to be most important in determining how many parasitoid larvae can develop on a host, and seems to have a lesser role in wasp development itself (Rivers & Denlinger, 1995b). If the site of parasitism is restricted to either the anterior or posterior body regions of *S. bullata*, an elevation in the hemolymph and fat body lipid content was apparent, but was most pronounced in posteriorly-parasitized flies. Despite differences in host lipid content, anteriorly and posteriorly parasitized flies yield the same number of parasites (Rivers & Denlinger, 1994b). These findings seem to conflict with the suggestion of Rivers & Denlinger (1995b) that more host fat equals more parasites. This apparent contradiction may be due to female wasps possessing a finite number of mature eggs available for oviposition during the 6 h time interval that *N. vitripennis* was presented fly hosts. When using young pharate adults of *S. bullata*, the preferred host age and fly species for *N. vitripennis* (Rivers & Denlinger, 1995a), female wasps laid the maximum number of eggs, regardless of the host body region used for parasitism. Thus, no further increase in fecundity would be observed despite site-specific differences in host lipids following venom injection.

Wasp larvae reared on posteriorly-parasitized flies contained more extractable lipid as second and third instars than did those parasitoids developing on flies with less fat (anteriorly-parasitized). These results imply that the feeding parasitoids deposit dietary fat directly into their tissues. If found to be true, the lipid profile (composition) of *N. vitripennis* would be expected to closely resemble that of the flesh fly host, and should change when using different fly species as hosts.

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