Parasites as The Cause of High Incidence of Non-Viable Fly Puparia at Animal Farms

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Our recent study of fly parasites at animal farms on Oahu (Toyama and Ikeda 1974), revealed that a large percentage of field collected fly pupae was dead from unknown causes. Among the possible causes of mortality, the parasites were suspected because of their method of host-feeding. Almost all major fly parasite species on Oahu inflict a wound on the host pupa with their ovipositor to feed on the exuding body fluids (Clausen 1962). The effects of parasite host-feeding on fly pupal mortality as reported by Edwards (1954), Wylie (1958), and Clausen (1962) were inconclusive. Edwards and Wylie observed no ill effects from host-feeding while Clausen cited a study that reported some fatalities. This study was undertaken to resolve whether parasites were responsible for these dead fly pupae, and if so, to determine how they were killed.

MATERIALS AND METHODS

Host species used were the major nuisance flies: *Musca domestica* Linnaeus, (Muscidae); *Musca sorbens* Weidemann, (Muscidae;) *Phaenicia cuprina* Wiedemann, (Calliphoridae); and *Chrysomya megacephala* (Fabricius), (Calliphoridae).

Three sampling sites were used from June 1974 to March 1975: a poultry farm at Haleiwa, Oahu which had an abundance of *M. domestica* in wet manure; a dairy at Kawailoa, Oahu where *M. sorbens* larvae were found in fresh untrampled dung pats; and a poultry farm at Ewa, Oahu which had many undisposed dead hens containing *P. cuprina* and *C. megacephala* larvae.

The effect of parasites upon viability of fly pupae was determined by comparing mortality rates of parasite-exposed and parasite-excluded host samples. Field collected larval hosts and their breeding medium were placed in shallow wooden boxes and exposed to parasites present at animal farms for three days after pupation. Parasite-excluded sample boxes were covered with fine-mesh organdy cloth.

To substantiate results of the field study above, a similar experiment was conducted in the laboratory. Five species of hymenopteran parasites were used: *Spalangia endius* Walker, (Pteromalidae); *Spalangia cameroni* Perkins, (Pteromalidae); *Muscidifurax raptor* Girault and Sanders, (Pteromalidae); *Dirhinus luzonensis* Rohwer, (Chalcididae); and *Eucoila impatiens* Say, (Cynipidae). A gravid parasite was placed in a cloth-covered plastic cup into which ten laboratory-reared hosts were added daily for five days. Eight replicates, consisting of paired test and control samples, were made for each parasite species. *M. domestica pupae* were used as hosts for all parasites except *E. impatiens*. *P. cuprina* larvae were used for *E. impatiens* because this parasite oviposited in the host larva and emerged

from the puparium. All adult parasites in the cups, except *E. impatiens,* fed upon host body fluids. Whether *E. impatiens* also fed on host body fluids was not known. However, our observations indicated that their fecundity was not impaired when provided only water. Moistened dog food used as nourishment for *P. cuprina* larvae provided water for *E. impatiens.*

The following possibilities were examined to determine how the hosts were killed by parasites: (1) mechanical killing by ovipositor mutilation during host feeding; (2) microbial infections caused by ovipositor contamination during host feeding; and (3) superparasitism, where both parasites fail to develop because of insufficient food.

The effect of ovipositor mutilation on host viability was determined by observing whether flies emerged from host pupae that were fed upon by parasites. Other pupae that were fed upon were kept for two days to allow ovipositor caused lesions to develop before being dissected for evidence of fatal lesions. Puparia were kept in Peterson's fluid (Peterson 1959) to harden their contents before dissection.

Host puparia penetrated by an ovipositor were observed to determine whether parasitization or fatality always occurred from ovipositor probing. A gravid *S. endius* and a puparium of *M. domestica* were kept in a vial until ovipositor penetration of the puparium was observed. These ovipositor-penetrated puparia were removed and held until flies or parasites emerged. Daily unused puparia were kept as controls.

Superparasitism and ovipositor-caused secondary microbial infections were determined by dissecting all puparia that were parasitized or nonviable from the field trials (Table 1) and controlled parasitism experiments (Table 2). The number of *M. domestica* puparia dissected was lower than those in Table 1 because one sample was accidentally discarded.

Whether hosts were killed by parasites that subsequently died during their larval instars was considered when dissection of non-viable host puparia revealed many dead parasite imagos within the puparia. Because putrefaction negated identification of dead parasite larvae in the above study, fresh, parasite-exposed, non-viable puparia were dissected to detect these larvae. Five female *S. endius* were placed in containers with 200 twelve hours old *M. domestica* puparia for three days. Following eclosion, the remaining non-viable puparia from three replicates were divided into two samples. One sample was immediately preserved in Peterson's fluid before being dissected and the other was kept to allow the parasites to emerge. Parasitization rates of both samples were compared to determine whether a greater rate occurred in the dissected sample.

The amorphous condition of the decayed contents of many dissected puparia suggested that freshly formed pupae undergoing histolysis were more susceptible to dehydration and secondary microbial infections from ovipositor penetration. To test this hypothesis, the parasites, *Mormoniella vitripennis* Walker (Pteromalidae) and *S. endius* were placed in containers and alternately exposed for twelve hours to freshly formed and older *M. domestica* puparia. Twenty host puparia were used for each exposure to five female parasites of each species. 1-12 and 12-36 hours old puparia were used. The same parasites were used for the entire test period.

Results

Comparison of mortality rates of parasite-exposed and unexposed host pupae at animal farms (Table 1) showed significantly higher rates only for *M. domestica* and *M. sorbens*. The lack of significant difference for *P. cuprina* and *C. megacephala* was not unexpected; these calliphorids were parasitized mostly by *E. impatiens*, a parasite that does not attack the host in the pupal stage.

TABLE 1. *Effect on Viability of Fly Pupae Exposed to Parasites Present at Animal Farms*

Host Species		Viable Pupae (%)	Parasitized Pupae (%)	Dead Pupae (%)	Total Sample
M. domestica	exposed	41.6	33.8	24.6*	8,633
	unexposed	92.7	0.0	7.3	9,460
M. sorbens	exposed	6.1	46.4	47.5**	1,021
	unexposed	95.3	0.0	4.7	13,478
P. cuprina	exposed	77.1	12.8	10.1	5,860
	unexposed	96.2	0.0	3.8	7,674
C. megacephala	exposed	74.6	4.1	21.3	2,714
	unexposed	97.2	0.0	2.8	1,922

* Significant $0.05 < P > 0.01$
** Highly Significant $P > 0.001$

This lack of significant difference, and the low unparasitized dead pupae rate for *E. impatiens*, in table 5, could be considered as further indication of a relationship between non-viable host puparia and ovipositor mutilation. Results of the controlled parasitization experiment (Table 2) corroborated the findings of the field study (Table 1). These two studies indicated that fly pupae were being killed by parasites.

Flies emerged from a few pupae that were fed upon by parasites. This viability demonstrated that ovipositor mutilation was not invariably fatal to the host. Puparia dissected several days after the pupae were fed upon by *S. endius* showed no apparent lesions that could cause death of the pupae. *D. luzonensis*, a much larger species than *S. endius*, caused lesions that still had remnants of feeding tubes attached. These lesions appeared superficial; however, the higher dead pupae rate for *D. luzonensis* in Table 5 could indicate that some fatalities had occurred from ovipositor mutilations.

Results in Table 3 showed 13.4% host puparia still viable. This indicated that ovipositor probing of the host puparium did not always result in parasitization or fatality. Whether these flies survived host feeding, nonviable parasite eggs, or ovipositor probing without egg deposition was not determined in this study.

TABLE 2. *Effect of Parasites on Viability of Fly Pupae*

Parasite Species	Host		Viable Pupae (%)	Parasitized Pupae (%)	Dead Pupae (%)	Total Sample
S. endius	*M. domestica*	exposed	5.8	67.7	26.5**	400
		unexposed	94.6	0.0	5.4	400
S.cameroni	*M. domestica*	exposed	17.3	56.7	26.0**	400
		unexposed	90.5	0.0	9.5	400
M. raptor	*M. domestica*	exposed	28.5	44.3	27.2**	400
		unexposed	91.0	0.0	9.0	400
D. luzonensis	*M. domestica*	exposed	32.3	34.7	33.0**	400
		unexposed	96.0	0.0	4.0	400
E. impatiens	*P. cuprina*	exposed	16.2	62.8	21.0**	400
		unexposed	98.0	0.0	2.0	400

** Highly Significant P > 0.001

Dissection of non-viable host puparia showed most contents to be putrefied; others contained dead parasites, or showed evidence ot multiple parasitism. Multiple parasitism, although high (Table 4), did not appreciably affect the parasitization rate because the principal second parasite, *M. raptor,* in Muscidae, or *M. vitripennis,* in Calliphoridae, usually survived. Dead parasites in dissected puparia were usually fully developed except for decaying portions of unsclerotized integument. Evidence of superparasitism was found in only two host puparia. In both instances, the parasites were imagines of *S. endius.*

TABLE 3. *Effect on Viability of* M. domestica *Pupae after Ovipositor Penetration of Puparia by Parasite* S. endius

	Viable Pupae (%)	Parasitized Pupae (%)	Dead Pupae (%)	Total Sample
Penetrated puparia	13.4	66.0	20.6**	306
Unpenetrated	96.3	0.0	3.7	1,127

** Highly Significant P > 0.001

The possibility that dead pupae were killed by parasites that subsequently died during their larval instars was not substantiated. Results in Table 6 showed no significant difference in parasitization rate between the dissected and undissected samples.

Results from the study to determine whether ovipositor punctures in the host integument during the early stages of metamorphosis caused higher host pupal mortality was inconclusive (Table 7).

TABLE 4. *Contents of Dissected Non-Viable Fly Puparia Exposed to Parasites Present at Animal Farms* (Table 1)[1]

Host Species	Exuviae of Viable Parasites (%)	Dead Parasites (%)	Unparasitized Super-Parasitized (%)	Unparasitized Dead Pupae (%)	Total Sample	Multiple Parasitism[2] (%)
M. domestica	63.9	7.8	0.0	28.3	3,632[3]	2.5
M. sorbens	53.9	10.0	0.0	36.1	959	4.5
P. cuprina	55.8	9.8	0.0	34.4	1,341	18.8
C.megacephala	16.1	1.7	0.0	82.2	689	0.0

[1] Non-viable Puparia = parasitized pupae + unparasitized dead pupae
[2] Multiple parasitism computed separately because it did not affect parasitization rate
[3] One sample accidentally discarded

TABLE 5. *Contents of Dissected Non-Viable Fly Puparia Exposed to Parasites* (Table 2)[1]

Parasite Species	Exuviae of Viable Parasites (%)	Dead Parasites (%)	Unparasitized Super-Parasitized (%)	Unparasitized Dead Pupae (%)	Total Sample
S. endius	71.9	10.1	0.0	18.0	377
S. cameroni	68.6	6.0	0.0	25.4	331
M. raptor	61.9	9.8	0.0	28.3	286
D. luzonensis	51.3	16.2	0.0	32.5	271
E. impatiens	74.9	20.3	0.0	4.8	335

[1]Non-Viable = parasitized + unparasitized dead pupae

TABLE 6. *Determination of Parasite Mortality During Larval Instars. Number of* S. endius *Larvae Found in Dissected Half of* M. domestica *Puparia Sample Compared with Parasites Eclosing from Other Half.*

	Parasitized (%)	Dead-Unparasitized (%)	Total Sample
Dissected Puparia (larvae)	62.8	37.2	253
Undissected Puparia (adults)	68.1	31.9	254
Control	0.0	9.4	502

TABLE 7. *Susceptibility of Freshly Formed Puparia to Fatalities from Ovipositor Mutilation by Pupal Parasites.* M. domestica *Puparia Kept at Average Ambient Temperature of* 27.8°C. *and* 60.6% R.H.

Parasite Species	Pupae Age (hours)	Viable Pupae (%)	Parasitized Pupae (%)	Dead Pupae (%)	Total Sample
M. vitripennis	1-12	31.5*	33.8	34.7**	400
	12-36	5.0	77.8	17.2	400
S. endius	1-12	27.9*	55.8	16.3	240
	12-36	17.5	67.5	14.8	240

* Significant $0.05 < P > 0.01$
** Highly Significant $P > 0.001$

Discussion

The data on *M. domestica* in Table 4, after being adjusted by including the natural mortality rate in Table 1, summarizes the probable causes of host pupal mortality in the field: (1) viable parasites, 63.9%; (2) dead parasites, 7.8%; (3) dead pupae caused by ovipositor penetration of the host integument, 21.0%; and (4) natural host mortality, 7.3%.

Results clearly indicated that parasites caused the high host pupal mortality. However, how they caused these mortalities was not as apparent. Evidence for mortality from ovipositor-caused microbial infections was inconclusive because the appearance of remains in dissected dead puparia from control samples were similar to those in the parasitized samples. The contents of dissected non-viable host puparia appeared as : putrefied remains; hollow, with a thin powdery material coating the inner surface of the puparium; amorphous dehydrated pellets; and mummified adult flies. Dehydrated pellets, which were absent from dissected control puparia, were the only type of remains that could be linked to the parasites. These straw-colored, amorphous pellets, which did not appear to have saprophytic microorganisms involved, may have resulted from uninfectious punctures that caused fluid loss and subsequent dehydration. Dehydrated pellets were the least abundant form of puparia contents in all host samples dissected.

M. raptor, the most common second parasite in multiple parasitism of Muscidae, was detected in the puparium by the presence of its meconium with exuviae attached. Puparia dissection showed that *M. raptor* was the only species that would parasitize almost fully developed flies.

M. vitripennis, a gregarious parasite, was the common second parasite of multiple parasitism in Calliphoridae. Multiple parasitism was detected by the presence of exuviae and either a body of an adult *E. impatiens* or its chalky white remains. This white material was the parasite's waste that would eventually have been ejected as meconium.

Dissection of *P. cuprina* puparia with exit holes showed many to have numerous dead imagos of *M. vitripennis* within. These exit holes suggested that parasitization by this gregarious parasite usually resulted in survival of at least a few specimens. If successful parasitization usually occurs, then the high dead host pupae rate by *M. vitripennis* (Table 7) could be considered as further indication that hosts killed by parasites that had died during their larval instars was not a major cause of the high dead pupae rate.

The unusually low parasitization rate and high pupal mortality for *C. megacephala* in Table 4 appeared to have been caused by its unsuitability as a host for its major parasite *E. impatiens.* Exposure of 343 *C. megacephala* larvae to 20 pairs of *E. impatiens* resulted in 97.4% dead host pupae and only three viable parasites. The control sample, which was unexposed to parasites, had only 7.7% dead host pupae.

Because all parasites studied, except *E. impatiens,* deposit their eggs on the surface of the host within the puparium, accidental puncturing of the host integument, particularly of freshly formed puparia, is not unlikely. Edwards (1954) has noted that freshly formed fly puparia are unsuitable for oviposition because no space exists between the integument and

puparium wall during histolysis. However, the ability of *S. endius* to successfully parasitize freshly formed puparia (Table 7) suggested the existence of a space. We expected this space to occur at the puparium ends because of our observations that *S. endius* usually oviposited at these ends while *M. vitripennis* preferred the intersegmental notches (Edwards 1954). Dissection of 5 hours old puparia kept at 28°C revealed invaginations around the mouthhooks that created a space at the anterior end. Puparia were kept in Peterson's fluid before dissection.

The significantly higher viability rate of the 1-12 hours old pupae in Table 7 was believed to be caused by survivors of a greater frequency of accidental ovipositor punctures of the host integument during histolysis. A second attack by parasites on these previously rejected pupae was unlikely because of the rarity of superparasitism by solitary parasites in this study. The possibility that parasites preferred older puparia and avoided freshly formed puparia was discounted because of the 1.02 times higher host mortality caused by *M. vitrepennis* (Table 7) in freshly formed puparia.

Summary

Data indicated that parasites were the cause of the high incidence of fly pupal mortality observed during our previous studies of flies at animal farms on Oahu. Findings also indicated that the fly pupae were being killed by: (1) secondary microbial infections caused by ovipositor mutilation of the host integument during parasite host feeding or oviposition; (2) deaths of immature parasites in the host puparium; and (3) dehydration through loss of body fluids from ovipositor mutilation. As to other contributing factors, superparasitism was rare, and multiple parasitism, although high, did not affect the parasitization rate because the second parasite species usually survived.

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