Development of *Alouattamyia baeri* (Diptera: Oestridae) from Howler Monkeys (Primates: Cebidae) on Barro Colorado Island, Panama

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ABSTRACT The fecundity and development of larval stages of the cuterebrid bot fly *A. baeri* were studied in an unusual host, remotely related to the primary host. Third-instar *Alouattamyia baeri* (Shannon & Greene) removed from howler monkeys, *Alouatta palliata*, were allowed to pupate and then were cultured under controlled conditions. Eclosion occurred after 37.9 ± 0.4 (mean ± SE) (♂) and 38.2 ± 0.4 (♀) d at 26°C. Five-day-old females were mated using a tethered flight technique and oviposited on ridged filter paper. The total egg complement was 1,399 ± 243 (n = 2) eggs per female. Eggs were fully embryonated after incubation for 5 d at 26°C. Eggs hatched when warmed in the palm of the hand. Hatching of eggs from an individual batch was asynchronous. Newly hatched larvae would not penetrate intact skin on a rabbit, *Oryctolagus cuniculus* L. Larvae placed near the nares or on the ocular conjunctiva migrated rapidly from view. Warbles containing larvae were first observed on infested rabbits 5 d after infestation. Development of larvae proceeded until day 39 after infestation, when nearly mature 3rd instars were observed. None of the larvae survived to pupate.

KEY WORDS *Alouatta palliata*, *Alouattamyia baeri*, bot flies, Cuterebridae, host–parasite relationships

KNOWLEDGE OF THE development and host specificity of cuterebrids generally is limited to the economically important neotropical species *Dermatobia hominis* (L.) and to several species infesting rodents and lagomorphs in temperate regions (see review in Catts 1982). Only a few studies on neotropical cuterebrids (e.g., *Metacuterebra apicalis* Bau, *Rogenhofera bonaerensis* Brauer) have presented information on developmental biology (Leite and Williams 1988, Vignau and Zuleta 1991).

Larvae of the cuterebrid bot fly *Alouattamyia baeri* (Shannon & Greene) parasitize members of the genus *Alouatta* at various sites throughout the Neotropics (Milton 1996). There is substantial evidence that infestations in young monkeys are an important factor in their mortality (Milton 1996). This relationship appears to play a key role in regulation of population size in the closed howler monkey, *Alouatta palliata*, population on Barro Colorado Island, Panama (Milton 1996). Details of the larval development of this unique cuterebrid are not known, and this information is important in advancing our understanding of the host–parasite relationship and how it may affect host survival.

Host selection by *A. baeri* is apparently specific, because there are reports of *A. baeri* infestation in only one other monkey species (*Aotus trivirgatus*, Guimaraes 1971) and the occasional accidental infestation in man (Guimaraes and Coimbra 1982, Fraiha et al. 1984). This host specificity is also observed in other cuterebrids, particularly those parasitizing rodents and lagomorphs because the larvae are capable of completing development only in a narrow range of hosts (Baird 1971). Host specificity and the potential role of other hosts in cuterebrid biology is interesting because howler monkeys on Barro Colorado Island share the forest with 4 other nonhuman primate species. Assessing the developmental specificity of this cuterebrid species may help determine whether the absence of infestation in the other nonhuman primate species is a result of developmental specificity or the result of other causes (e.g., lack of contact with eggs and newly hatched larvae).

The objective of the current research was to define life cycle parameters of *A. baeri*, including egg and pupal developmental times, sex ratio, mating and oviposition behavior, and fecundity. In addition, host entry behavior of 1st instars and development in a convenient laboratory model, the rabbit, *Oryctolagus cuniculus* L. (New Zealand white strain), were investigated. Potential host range for this species of cuterebrid also was addressed by using the rabbit as a model host.

Materials and Methods

Howler monkeys were immobilized with Telazol (25 mg/kg) delivered by dart from a CO_2-powered gun. Immobilized monkeys were caught in a net and placed in a covered metal cage for transport to the laboratory. After a detailed examination and specimen collection, each monkey was placed in a covered cage, allowed to recover, and then returned to its troop of

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Table 1. Summary of rabbit (*O. cuniculi*) infestations with larvae of the howler monkey bot fly, *A. baeri*

Rabbit	No. larvae	Site	Days to 1st appearance	Max count
NV1	7	Nares	6	4
	8	Conjunctiva	6	4
NV7	10	Nares	8	6
NV8	6	Conjunctiva	8	2

origin (collections and export and import of specimens were under permit No. 2565 and 37-92, Panama, and permit No. 150909, Canada, respectively).

Monkeys were examined thoroughly for lesions and representative larvae from several hosts were expressed gently from the warble. The developmental stage of each recovered specimen was noted and each bot was weighed before being placed individually either into 12-cm-diameter pots containing 15 cm of soil taken from a nearby rainforest or into plastic vials containing sterilized wood chips. A sample of the larvae was weighed daily and developmental changes noted. Larvae were held at ambient temperature (range, 24–29°C) and humidity (85–95% RH) for up to 10 d and then were maintained at 26°C, 85% RH, and a photoperiod of 12:12 (L:D) h until development was completed.

Three to 5 d after eclosion, we attempted to mate flies by using the technique of Weintraub (1961). Mating attempts took place under full-spectrum illumination at 26°C and 85% RH. Successfully mated females were presented with either leaves of *Ficus* spp. or ridged filter paper as an oviposition substrate. Eggs were incubated at 26°C and 85% RH. Exposure of eggs to both rapid warming, by placement of a culture dish in the palm of the hand, and human breath stimulated hatching.

Newly hatched larvae were transferred to the nares, ocular conjunctiva, or shaved skin of 4 rabbits (Table 1). Before larval exposure, each rabbit was anesthetized by intramuscular injection of ketamine (35–50 mg/kg) and xylazine (5–10 mg/kg). Larvae were observed until they died or they disappeared from sight. Rabbits were examined 2 h after infestation and then daily for up to 45 d.

Animals were handled and cared for under the guidelines of the Canada Council for Animal Care.

Histology. To clearly understand the feeding biology and the host–parasite relationship within the warble, histological observations were made on both the midguts of 3rd instars and the warble within which they were contained. Several mature 3rd instars were injected with ≈1.5 ml of neutral buffered formalin immediately following recovery from the host. Midguts were removed, washed in water, dehydrated in a graded series of ethanol, and embedded in HistoResin (LKB, Bromma, Sweden). Sections (2 μm) were cut with glass knives, mounted on glass slides, and stained with toluidine blue.

Warbles and a small amount of adjacent skin were removed surgically from anesthetized rabbits and howler monkeys. These tissues were fixed in 10% neutral buffered formalin, embedded in Paraplast, and sectioned (5 μm). Sections were stained with haematoxylin and eosin.

Results

Fifty 3rd instars were collected from 8 individual monkeys ranging in age from 3 mo to 15 yr. The larvae were black and motile at the time of collection. Forty-six (92%) of the larvae pupated within 1–3 d (mean ± SEM, 2.05 ± 0.55 d). Following culture under controlled conditions, 24 (52.2%) flies (15 males, 9 females) emerged. The sex ratio was not significantly different from 1:1 ($\chi^2 = 1.042$). The developmental period of the 2 sexes was not significantly different ($t = 0.625$, df = 21, $P = 0.539$), with males requiring a mean ± SEM of 37.9 ± 0.4 d to complete development whereas females required 38.2 ± 0.4 d.

The flies were large and black and very active fliers. Females were distinguished easily from males by having the greater interocular distance (Fig. 1). When alive, male flies also were distinguished by the presence of a distinct red stripe running vertically across each eye (Fig. 1). The red stripe disappeared shortly after death of the fly.

Larvae from which female flies emerged weighed significantly more than those from which males emerged, both at collection and on the day of pupation: 2.82 ± 0.33 versus 2.28 ± 0.58 ($t = 3.704$, df = 22, $P < 0.002$) at the time of collection, and 2.19 ± 0.25 versus 1.77 ± 0.48 ($t = 3.52$, df = 22, $P < 0.002$) on the day of pupation. Mean ± SEM weight loss between collection and pupation was 22.2 ± 2.04% for both sexes.

Three female flies were mated successfully between 3 and 5 d after eclosion. These flies would not oviposit on *Ficus* spp. leaves; however, all flies oviposited when placed on the ridged filter paper. These females oviposited mean ± SEM of 262.7 ± 149.4 eggs (range, 53–552). Eggs were attached firmly to the substrate and generally were deposited along the ridge of the fold in long rows. Two flies that did not oviposit were dissected and had a mean ± SEM complement of 1,399 ± 243 eggs.

Fully embryonated eggs first were observed after 5 d culture at 26°C. Larvae emerged from the eggs via the operculum following exposure to breath and warming in the palm of the hand. Only a few larvae from each batch of eggs hatched after each exposure to these conditions.

Histology. Histological observations on the midgut of 3rd instars showed the presence of a well-developed peritrophic membrane surrounding the food bolus (Fig. 2a). Within the food bolus there were numerous host white blood cells (Fig. 2b), including granulocytes.

Development in Rabbits. Larvae ($n = 10$) placed on shaved rabbit skin did not penetrate the unbroken skin and were no longer motile after 15 min. First instars placed on the nares or on the ocular conjunctiva rapidly migrated from view within 5 min (Table 1). The rabbits did not exhibit apparent adverse reactions

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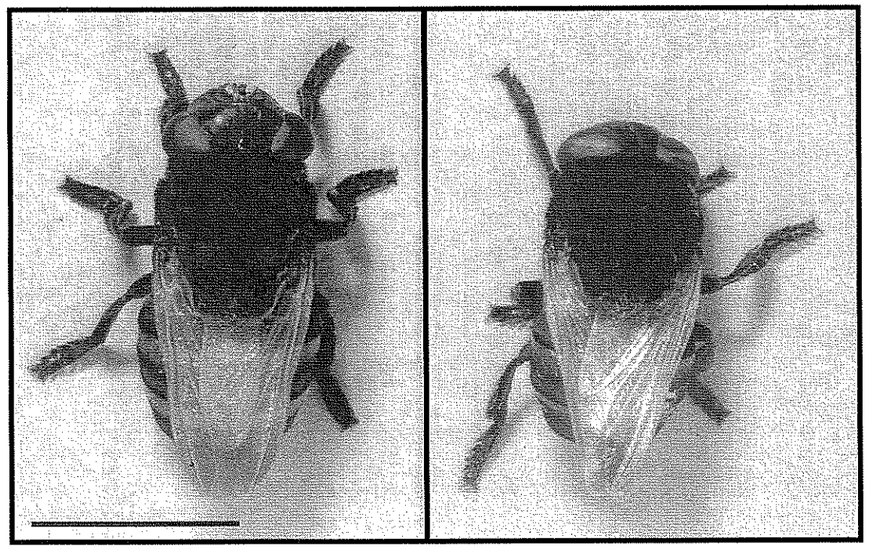


Fig. 1. Two newly emerged *A. baeri*. Note the size difference between male (right) and female (left) and the larger interocular distance in the female. The male has a distinct red band running vertically across each eye. Bar = 1 cm.

to entry of the larvae, and no symptoms were observed until warbles were first observed between 6 and 8 d after infestation (Table 1). The 1st warbles were observed on the neck and shoulder region of the rabbits (Fig. 3 a and b). Up to 6 larvae were observed on a single infested rabbit (Table 1), with the majority being located on these areas, although occasional warbles were noted on the ventral abdomen or groin.

Warbles on the rabbits were inflamed and a purulent exudate usually was evident around the breathing holes. The warbles were apparently irritating to the rabbits as they were observed to actively groom the sites. Histological examination of excised warbles showed a dramatic infiltration of eosinophils (Fig. 4a) when compared with those removed from a howler monkey (Fig. 4b).

Development of the larvae within the rabbits was monitored by removal of individuals at various times after infestation (Fig. 5 a and b). These observations are summarized in Table 2. The larvae that exited 36 d

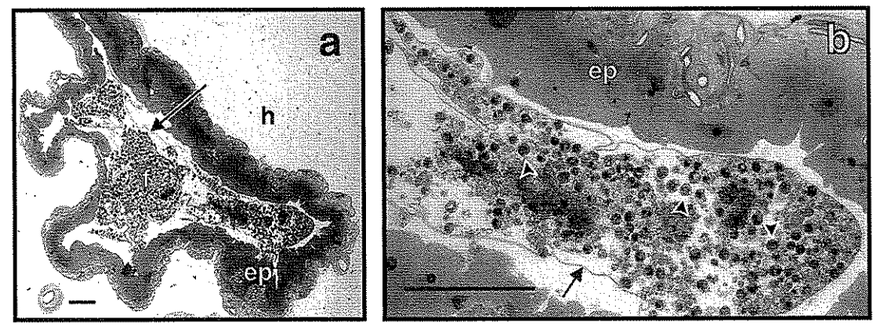


Fig. 2. Light micrographs of a semi-thin sections through the midgut of a 3rd-instar *A. baeri* removed from a howler monkey, *A. palliata*. (a) Low magnification showing the midgut epithelium (ep) and the well-defined peritrophic membrane (arrow) surrounding the food bolus; bar = 1 mm; and (b) higher magnification view of the midgut showing the epithelium (ep), the peritrophic membrane (arrow), and the food bolus, containing large numbers of host white blood cells (arrows). Bar = 1 mm.

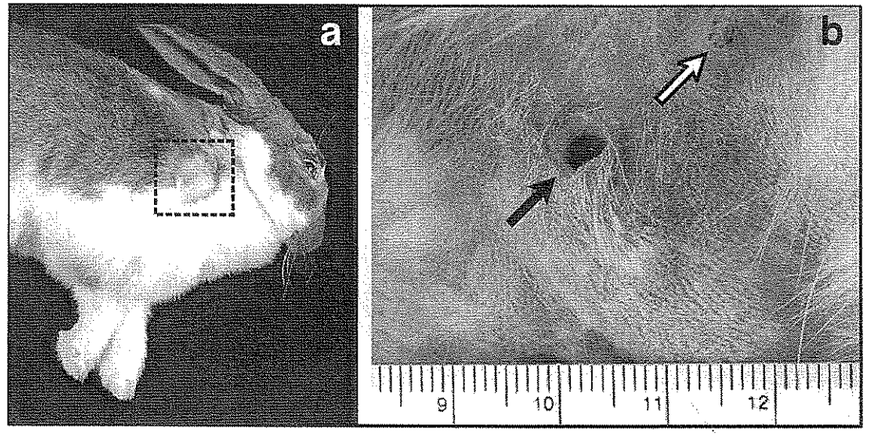


Fig. 3. Photographs of rabbits artificially infested with larvae of *A. baeri* (21 d after infestation). (a) Side view of infested rabbit with warble on the neck region. Hair has been removed from the warble. (b) Close-up view of warble showing the breathing hole (black arrow). The healing breathing hole of a warble in which another larvae has died is also present (white arrow) (smallest increment = 1 mm).

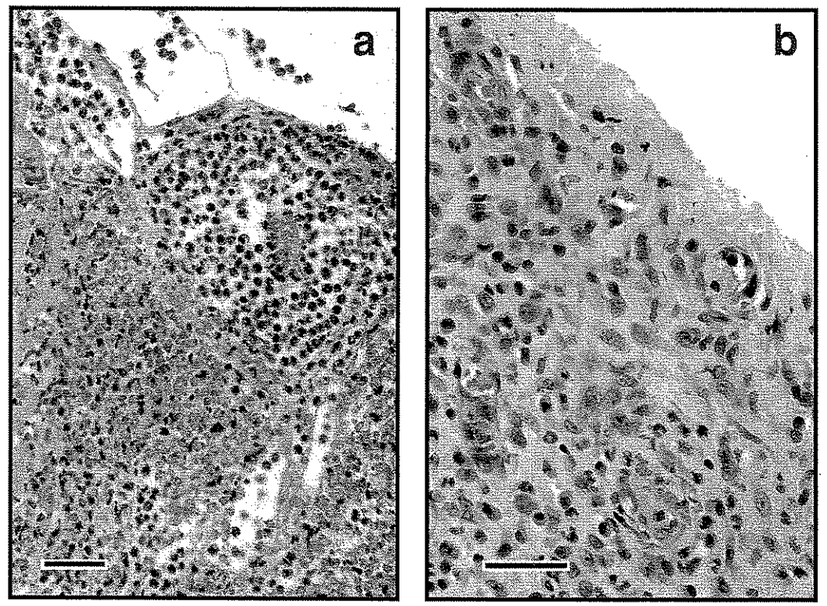
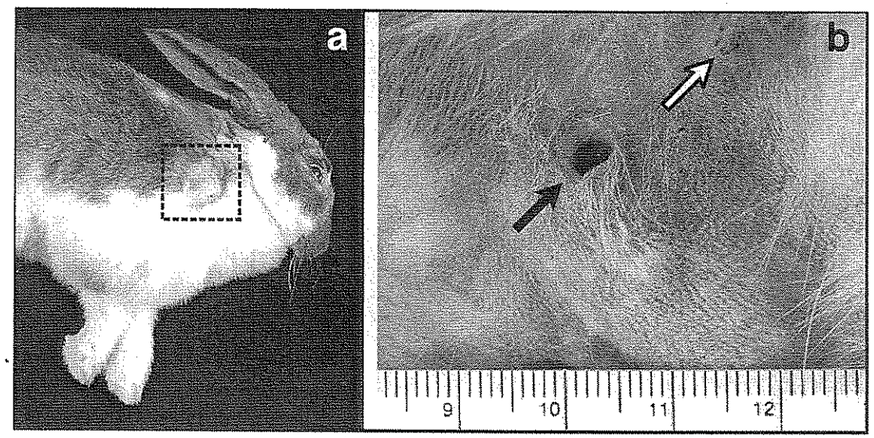


Fig. 4. Light micrographs of section through warbles induced by *A. baeri*. (a) Section through a warble from an artificially infested rabbit. The region occupied by the larva is in the upper right. Note the invasion by polymorphonuclear white cells, particularly eosinophils, and the disruption of the inner surface. Bar = 0.1 mm. (b) Section through a warble from a howler monkey. The region occupied by the larva is in the upper right. Note the relative absence of polymorphonuclear cells and the intact inner surface. Bar = 0.01 mm.



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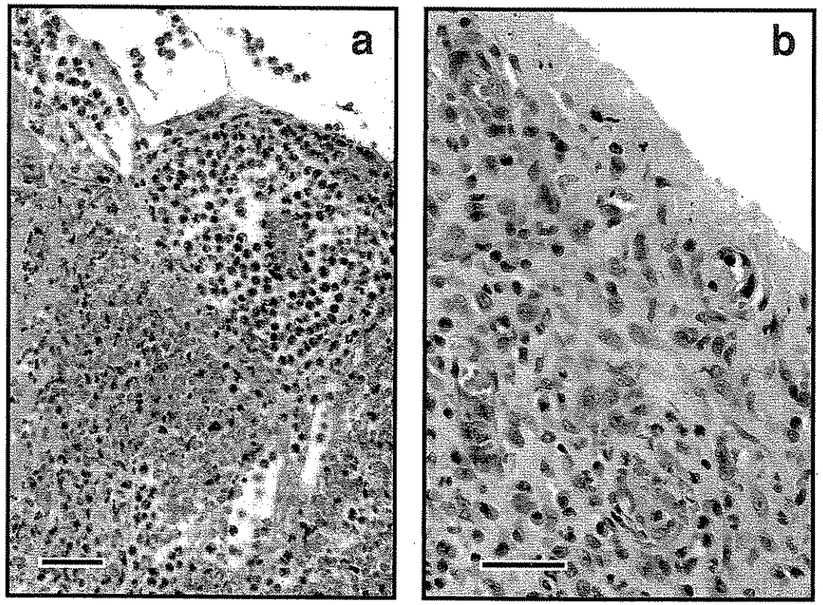

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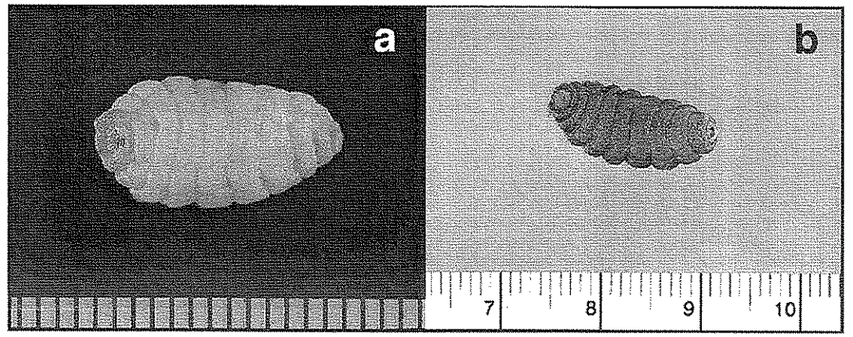

Fig. 5. Third-instar larvae removed from rabbits artificially infested with *A. baeri*. (a) Ventral view of cream-colored larva removed 21 d after infestation. (b) Ventral view of light brown larva removed 28 d after infestation (smallest increment = 1 mm).

after infestation were fully black but were apparently eaten by the rabbits before they could pupate. The single larva collected 37 d after infestation weighed 1.9 g but did not pupate successfully.

Discussion

Pupal Development and Adult Biology. Mature cuterebrid larvae and pupae that develop into female flies tend to be heavier than males (Haas and Dicke 1958, Baird 1971, Baird 1972). A similar pattern was observed in the current study. The difference in weight is usually a result of the egg complement, as both male and female oestrids emerge from the puparia with substantial fat bodies that are used to sustain mating and oviposition search flights (Anderson et al. 1994). In contrast, Leite and Williams (1988) noted no difference in pupal weights between the 2 sexes of the tropical rodent bot, *Metacuterebra apicalis* (Guérin-Menéville).

The pupal developmental period reported here was shorter (≈38 d versus 41–48 d) than that noted by Milton (1996) for 2 pupae that were reared under ambient conditions on Barro Colorado Island. Also, the pupal development of males in the current study was slightly shorter than that of females, but the difference was not significant. Milton (1996) previously noted a shorter period for a male fly than for a female fly (41 versus 48 d). In other cuterebrids the males tend to have a shorter pupal developmental period than females (Baird 1975, Catts 1982). It has been postulated that this adaptation allows the males time to gather at aggregation sites prior to the arrival of females (Catts 1982).

Table 2. Developmental stage of larvae removed from rabbits (*O. cuniculi*) artificially infested with larvae of *A. baeri*

Days after infestation	Instar	Cuticular coloration
17	2nd	White
20	3rd	White
28	3rd	Light brown
36	3rd	Black
	3rd	Black
37	3rd	Black, did not pupate

The sex ratio for cuterebrid flies is characteristically 1:1 (Catts 1982). In this study the ratio was 1:1.67 (♀:♂), but was not significantly different from 1:1. Other studies have reported highly skewed sex ratios. Smith (1975) reported a male-biased ratio for *C. approximata* Walker and speculated that such a skewed sex ratio was required to offset high male mortality at aggregation sites. Catts (1982) supported this conjecture. The sex ratio for the tropical rodent bot was unusual in that it was biased in favor of females (Leite and Williams 1988).

This is the first report of male *A. baeri* exhibiting a red eye stripe. Presumably, previous descriptions (Shannon and Greene 1926, Guimaraes 1989) relied on dead, pinned specimens in which the character was absent. The presence of red eye spots or a red eye stripe has been reported in male cuterebrids of lagomorphs of North America (Sabrosky 1986) and in males of the South American *M. apicalis* from a mouse, *Oryzmys subflavus* (Leite and Williams 1988). Of these reports, *Cuterebra ruficrus* (Austen) and *M. apicalis* were the only species in which a solid red stripe was present (Baird 1972). All other species have paired red eye spots (e.g., *C. lepivora*, Catts and Radovsky 1962, and *C. jellisoni* Curran (Baird 1971).

In our study the females were receptive to mating 3–5 d after eclosion and oviposited viable eggs shortly after mating. Scholl (1991) indicated that adult *C. fontinella* Clark required 5 d for development of fully mature eggs capable of being fertilized. The somewhat more rapid development in *A. baeri* may reflect differences in culture conditions (alternating 27–15°C versus constant 26°C). The fecundity of female *A. baeri* in our study was in the middle of the range reported for other cuterebrids (Table 3), being greater than that reported for *C. jellisoni* Curran, similar to that of *C. lepisculi* Townsend and *C. ruficrus*

Table 3. Fecundity, larval developmental period, host, and host specificity in a variety of lagomorph and rodent bot flies

Species	Eggs/♀	Larval development, d	Host	Host specificity
C. horripilum (=*cuniculi* Clark)	2,297		*Sylvilagus* sp.	High
C. ruficrus	1,727 (1,475–1,884)	74	*Lepus californicus*	High
C. jellisoni	1,006 (524–1,260)	36	*L. californicus*	High
C. lepusculi	1,286 (800–1,385)	27	*Sylvilagus* sp.	High
C. tenebrosa		27–39	*Neotoma* spp.	High
M. apicalis	2,714 (2,507–2,921)	22–31	*Oryzomys subflavus*	Moderate

(Austen), and less than that for *C. horripulum* = *cuniculi* (Clark).

Histology. The presence of a peritrophic membrane in 3rd instars was consistent with observations on other oestrid larvae (Boulard 1969). Similarly, the presence of host granulocytic white blood cells within the food bolus has been described for cattle grubs (Wolfe 1959). In cattle grub larvae the peritrophic membrane was absent in 1st instars but was present in 2nd and 3rd instars (Boulard 1969). This change in cattle grubs was coincidental with the change in food type from the liquid soup of the 1st instars to the more solid food ingested by the later 2 instars. The later instars use the host response to their advantage, residing within the granulmatous warble and using the cellular infiltrate produced by the host as a food source.

Development in Rabbits. The inability of 1st instars to penetrate unbroken rabbit skin was not unexpected considering the mode of entry of other cuterebrid larvae into their hosts (Catts 1982). The rapid entry of larvae into the nares and beneath the ocular conjunctiva indicates that these sites were favored over other sites.

The range in developmental periods for rodent bot flies is 19–42 d, whereas the range for lagomorph species is 26–73 d (Catts 1982). Rabbit infestations in our study required a developmental period (37–39 d) that was at the upper end of the rodent range and closer to the midrange for rabbit species. The detection of 3rd instars 20 d after infestation in rabbits was consistent with Catts' (1982) suggestion that molting to the 3rd instar occurs between 14 and 19 d in most cuterebrids.

The purulent exudate from the rabbit warbles appeared to be more intense and more consistent than on naturally infested howler monkeys (Milton 1996). The differences seen between the discharge observed on rabbits and that seen on howler monkeys was consistent with the differences in histology of the warble tissues. The large number of eosinophils infiltrating the warble in rabbits probably accounts for the irritation observed.

Although the development of *A. baeri* was not completed in rabbits, we suggest our study represents one of the few cases where even partial development was supported in a host not closely related phylogenetically to the primary host. This observation is of interest because there have been no reports of bot infestation in monkey species that share the arboreal habitat with howler monkeys on Barro Colorado Island. One particular explanation for the absence of accidental infestations of other closely related monkey species is that oviposition by *A. baeri* females occurs in locations where there is little or no opportunity for these species to come into contact with the eggs. Oviposition sites for *A. baeri* have not been described.

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Table 3. Fecundity, larval developmental period, host, and host specificity in a variety of lagomorph and rodent bot flies

Species	Eggs/ ♀	Larval development, d	Host	Host specificity
C. horripilum (=*cuniculi* Clark)	2,297		*Sylvilagus* sp.	High
C. ruficrus	1,727 (1,475–1,884)	74	*Lepus californicus*	High
C. jellisoni	1,006 (524–1,260)	36	*L. californicus*	High
C. lepusculi	1,286 (800–1,385)	27	*Sylvilagus* sp.	High
C. tenebrosa		27–39	*Neotoma* spp.	High
M. apicalis	2,714 (2,507–2,921)	22–31	*Oryzomys subflavus*	Moderate

(Austen), and less than that for *C. horripulum* = *cuniculi* (Clark).

Histology. The presence of a peritrophic membrane in 3rd instars was consistent with observations on other oestrid larvae (Boulard 1969). Similarly, the presence of host granulocytic white blood cells within the food bolus has been described for cattle grubs (Wolfe 1959). In cattle grub larvae the peritrophic membrane was absent in 1st instars but was present in 2nd and 3rd instars (Boulard 1969). This change in cattle grubs was coincidental with the change in food type from the liquid soup of the 1st instars to the more solid food ingested by the later 2 instars. The later instars use the host response to their advantage, residing within the granulmatous warble and using the cellular infiltrate produced by the host as a food source.

Development in Rabbits. The inability of 1st instars to penetrate unbroken rabbit skin was not unexpected considering the mode of entry of other cuterebrid larvae into their hosts (Catts 1982). The rapid entry of larvae into the nares and beneath the ocular conjunctiva indicates that these sites were favored over other sites.

The range in developmental periods for rodent bot flies is 19–42 d, whereas the range for lagomorph species is 26–73 d (Catts 1982). Rabbit infestations in our study required a developmental period (37–39 d) that was at the upper end of the rodent range and closer to the midrange for rabbit species. The detection of 3rd instars 20 d after infestation in rabbits was consistent with Catts' (1982) suggestion that molting to the 3rd instar occurs between 14 and 19 d in most cuterebrids.

The purulent exudate from the rabbit warbles appeared to be more intense and more consistent than on naturally infested howler monkeys (Milton 1996). The differences seen between the discharge observed on rabbits and that seen on howler monkeys was consistent with the differences in histology of the warble tissues. The large number of eosinophils infiltrating the warble in rabbits probably accounts for the irritation observed.

Although the development of *A. baeri* was not completed in rabbits, we suggest our study represents one of the few cases where even partial development was supported in a host not closely related phylogenetically to the primary host. This observation is of interest because there have been no reports of bot infestation in monkey species that share the arboreal habitat with howler monkeys on Barro Colorado Island. One particular explanation for the absence of accidental infestations of other closely related monkey species is that oviposition by *A. baeri* females occurs in locations where there is little or no opportunity for these species to come into contact with the eggs. Oviposition sites for *A. baeri* have not been described.

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