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RESEARCH

Evaluation of the Infestation Rate of *Blaesoxipha alcedo* in the Carrion Roller Scarab *Canthon cyanellus cyanellus* and Its Effect on Reproductive Behavior

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ABSTRACT. This study documents *Blaesoxipha alcedo* (Aldrich; Diptera: Sarcophagidae) parasitizing the necrophagous ball roller beetle *Canthon cyanellus cyanellus* LeConte collected over 2 yr, and evaluates the reproductive behavior of parasitized beetles. Up to 52% of the beetles collected in the field had been parasitized and exhibited modified sexual behavior. In general, 21% of the males and 24% of the females had been parasitized. The number of parasitized males peaked in August 2000 (52%), but that of females peaked in May 2001 (52%). The lowest percentage of parasitized males (7%) was recorded in September 2000, June 2001, and July 2001; for females, parasitism was lowest in June 2000 (10%). The larva of this sarcophagid fly penetrates the host through the tegumentary membrane, leaving a scar. The larvae feed on the gonads of male and female *C. c. cyanellus*, castrating them, causing the loss of their ability to recognize individuals of the opposite sex, preventing reproduction, and lowering overall reproductive success.

Key Words: Sarcophagidae, endoparasite, parasite-associated behavioral changes, Scarabaeinae

Some Sarcophaginae are parasitoids that infect a variety of arthropods, and of these, some have been used in forest and crop pest biological control programs (Pape et al. 2004, Scudder and Canning 2006, Salas-Araiza and Salazar-Solís 2009). However, because of their generalist nature, parasitoids can sometimes have adverse effects on native species that carry out important functions within the ecosystem (Boettner et al. 2000, Oberhauser et al. 2007).

In addition to causing high rates of mortality, one of the effects of the parasitoids on many insect species is the modification of their reproductive behavior (Uchida and Ehara 1953, Godfray 1994). In most cases, parasites partially or totally castrate the host, which then changes its reproductive behavior and alters its hormonal balance, and there are other modifications to the host's reproductive effort that favor the development of the parasitoid (Baudoin 1975, Beani 2006). With partial castration, the host has a chance of producing offspring before dying, whereas with total castration, reproduction becomes impossible (Bonds 2006).

The ball roller beetle, *Canthon cyanellus cyanellus* LeConte is a necrophagous species that lives in the tropical forests of the Americas (Halffter 1977, Favila 1993, Favila and Díaz 1996, Villalobos et al. 1998). This species feeds and breeds using the carcasses of small mammals, and its feeding and reproductive behaviors contribute to recycling nutrients back into the soil, as do other dung and carrion beetles of the subfamily Scarabaeinae (Nichols et al. 2008). Individuals of both sexes locate the food source by smell, and males also emit pheromones once at the carcass to attract a female and form a breeding pair (Halffter et al. 1983, Favila 2001). Sexual recognition is chemically mediated and occurs over a short distance on or near the carcass (Favila and Díaz 1996, Ortiz-Domínguez et al. 2006b). The male and female make head-to-head contact, extending their palps and antennae to touch the cuticle of the potential partner. If the beetles are of the same sex, they will fight for the food, but if they are of the opposite sex, they will make a ball from the carrion and roll it to a site about 2 m away from the food supply where they will bury it just below the surface for nesting (Favila and Díaz 1996, Favila 2001). Generally, the male rolls the ball and the female is transported on it. When the beetles are immature, they will fight with males and females for the food ball and roll it individually (Favila 1988).

In field collections that were carried out for population studies of *C. c. cyanellus* along a latitudinal gradient on the coastal plains of eastern Mexico, Ortiz-Domínguez et al. (2006a) found that some populations from the northern part of its distribution in Mexico were being parasitized by a species of Sarcophagidae that was identified as *Blaesoxipha alcedo* (Aldrich). To date, there has been no research on the effect of this parasite on the survival and reproductive behavior of *C. c. cyanellus*.

In this study, we analyze the percent infestation of the necrophagous ball roller beetle *C. c. cyanellus* by *B. alcedo* over two annual cycles in one of the locations where this parasitoid was detected. In addition, we determine the larval stages present within infected beetles and evaluate potential modifications of the host's reproductive behavior. We hypothesized that parasitized beetles would become unable to recognize individuals of the opposite sex, and unable to reproduce even in the presence of a normal mature individual of the opposite sex because *B. alcedo* eats the gonads of both males and females, producing a total castration of the host.

Materials and Methods

Evaluation of Infestation Rate in Field. Each month, from January 2000 to December 2001, 65 pitfall traps baited with squid were set on a cattle ranch near Papantla, Veracruz, Mexico (20° 25′ N, 97° 27′ W) for 48 h in order to capture live specimens of *C. c. cyanellus*. The specimens collected were kept in plastic containers with 2.5 cm of sterile soil and fed pieces of fish every third day in a rearing room (27 ± 1°C, 70 ± 10% relative humidity, and a photoperiod of 12:12 [L:D] h; Favila 1993). Specimens were kept in the rearing room until behavioral testing was carried out or until they had been killed by a parasitoid. Mortality associated with the presence of *B. alcedo* was recorded for every monthly capture.

***B. alcedo* Biology.** In 2000, a small scar was observed on the integument between the pronotum and the thorax of all beetles from which after death a third stage *B. alcedo* larva emerged through the pygidium plate. In 2001, thirty-five beetles, each of which had this scar on their

integument when collected, were killed by freezing (20 min, −20°C) for subsequent dissection. This was done to confirm the presence of the parasitoid inside the beetle and to determine the different stages of *B. alcedo* larval development. We maintained 20 beetles with scar on the integument between the pronotum and the thorax, until the third stage larvae eclosed from each beetle. The pupae were placed in a Petri dish until the emergence of the adults. All of the larvae collected were preserved in KAAD solution (toluene, acetic acid, alcohol, and dioxane) for 24 h and then stored in 70% alcohol. The adults were deposited in the Diptera collection of the Instituto de Ecología, A.C., Xalapa, Veracruz, Mexico.

Effect of the Parasitoid on the Reproductive Behavior of *C. c. cyanellus*. In a first test, we evaluated the frequency of acceptance or rejection of parasitized beetles ($n = 24$ males, $n = 19$ females) versus nonparasitized opposite-sex individuals raised in the laboratory ($n = 19$ males, $n = 24$ females). In each trial, a male–female pair was placed in an observation arena (150- by 15-mm Petri dish with filter paper) containing a food ball of artificial ground beef (0.8 g). If both beetles rolled the food ball for at least 15 min, this cooperative behavior was noted as acceptance. When an individual did not cooperate in rolling the food ball with the other beetle, or even fought for possession of the food, this behavior was noted as rejection. As controls, males and females collected in field with no scar on the integument between the pronotum and the thorax were put into quarantine to check that they had not been parasitized. They were then placed in observation arenas containing a food ball of artificial ground beef (0.8 g; $n = 20$ males, $n = 20$ females), and the observation protocol described earlier was conducted. All behavioral tests were conducted from 0900 to 1300 hours, which is the most active period for rolling in *C. c. cyanellus* (Favila and Díaz 1996). Parasitized beetles, though they demonstrated uncooperative behavior (see Results), were forced to nest together with laboratory-raised unparasitized specimens of the opposite sex in plastic containers with 2.5 cm of sterile soil in a rearing room (27 ± 1°C, 70 ± 10% relative humidity, and a photoperiod of 12:12 [L:D] h). Each pair was given food twice a week until nesting began or until the infected beetle died as a result of the parasitoid. For the control, 20 unparasitized pairs were also put to nest in the same way as the parasitized beetles.

Results

Evaluation of Infestation Rate in Field. Over 2 yr of study, a total of 1,779 specimens of *C. c. cyanellus* were collected: 946 males and 833 females. The average total percentage of parasitized individuals was 21% of the males and 24% of the females. The percentage of parasitized males peaked in August 2000 (52%), and in females the maximum was recorded in May 2001 (52%). The lowest percentage of parasitized individuals was 7% of the males during September 2000, June 2001, and July 2001, and 10% of the females in June 2000 (Table 1).

Table 1. Percentage of *C. c. cyanellus* parasitized by *B. alcedo* over two annual cycles (2000 and 2001) in Papantla, Veracruz, Mexico

	Males collected	% Males parasitized	Females collected	% Females parasitized
April 2000	99	43	65	20
May 2000	145	34	93	34
June 2000	80	14	68	10
July 2000	90	8	101	17
Aug. 2000	56	52	22	18
Sept. 2000	27	7	63	11
Oct. 2000	57	16	53	32
Mar. 2001	65	14	78	12
April 2001	71	18	84	33
May 2001	53	28	54	52
June 2001	90	7	32	13
July 2001	43	7	77	16
Aug. 2001	70	24	43	42
Total	946	21	833	24

***B. alcedo* Biology.** All of the beetles with a small scar on the integument had been parasitized by *B. alcedo* larvae ($n = 35$). Following dissection, 4 larvae were obtained at the first larval stage, 8 at the second stage, and 23 at the third stage, the latter housed in the pygidial plate of the beetles. The third-stage larvae of *B. alcedo* lodged themselves inside the pygidium and the beetle's gonads were absent, indicating that they had been eaten by the larvae. Ten pupae were incubated, and adults emerged after 14 ± 1 d (Fig. 1).

Effect of the Parasitoid on the Sexual Behavior and Reproductive Success of *C. c. cyanellus*. None of the parasitized beetles exhibited cooperative food-ball rolling behavior. Parasitized beetles rejected their partners, rolling alone, pushing them away with their heads, and preventing them from mounting the food ball. In contrast, all unparasitized pairs rolled the food ball cooperatively (Fisher's exact test, $P = \ll 0.0001$). All parasitized beetles that were forced to nest with laboratory-raised specimens of the opposite sex died within a maximum period of 7 d without producing offspring. In contrast, during the same 7 d, all of the control pairs nested and made a total of 51 brood balls, with 25 males and 26 females emerging as adults (100% survival).

Discussion

To our knowledge, this is the first report of a sarcophagid fly parasitizing the necrophagous ball roller beetle *C. c. cyanellus*. Monteith and Storey (1981) mention the presence of the diptera *Leptocera myrmecophila* Knab and Malloc, a phoretic fly that tunnels into the nest ball of the beetle *Cephalodesmius armiger* Westwood, but the actual relationship between these two species has not been studied in detail. Favila and Díaz (1996) mention that Phoridae flies attempt to lay eggs on carrion balls rolled by beetles, but did not find that they parasitize adult beetles.

We found that as much as 50% of the population of this species may be parasitized and consequently die. This is high considering that, for many years, our research group has collected live *C. c. cyanellus* along the coast of the Gulf of Mexico (Favila 1988, 2005; Halffter et al. 1992; Favila and Díaz 1996; Favila 2005; Ortiz-Domínguez et al. 2006a), and had never found a parasitized specimen in the populations of central and southern Veracruz state until now. Studies are therefore required to accurately determine the geographical distribution of the Sarcophagidae and of *B. alcedo* in particular, assess whether this parasitoid could affect other populations of *C. c. cyanellus*, and even other Scarabaeinae under natural conditions. The effect of this parasitoid on the ability of parasitized dung beetles to recycle dung, but especially carrion, also should be analyzed.

At the behavioral level, it is known that under natural conditions sexually mature male–female pairs of *C. c. cyanellus* show cooperative prenesting behavior, such as cutting and rolling a food ball together. In this study, we found that neither males nor females of reproductive age show this cooperative behavior when they are parasitized by *B. alcedo* larvae. In contrast, they display uncooperative behavior such as individual rolling, and pushing or even fighting with their partner for possession of the food. In the absence of parasitization, such uncooperative behavior is characteristic of immature males and females, or of adults of the same sex (Favila 1988). The absence of cooperative behavior in parasitized individuals was clearly related to the total castration of the host that modified the behavior of the beetle as a result of unknown hormonal changes. When parasitized beetles were placed together with unparasitized beetles of the opposite sex, no offspring were ever produced, also indicating that *B. alcedo* larvae totally castrate parasitized beetles.

Total or partial castration of the hosts by parasitoids is a phenomenon that could have beneficial effects as pest control (Greathed et al. 1994, Ramírez-Salinas et al. 2006). However, in this particular case, the parasitoid has a harmful effect that is detrimental to the reproductive success of *C. c. cyanellus*, a species that is beneficial to the ecosystem for its nutrient recycling capability. Our results indicate that *B. alcedo*

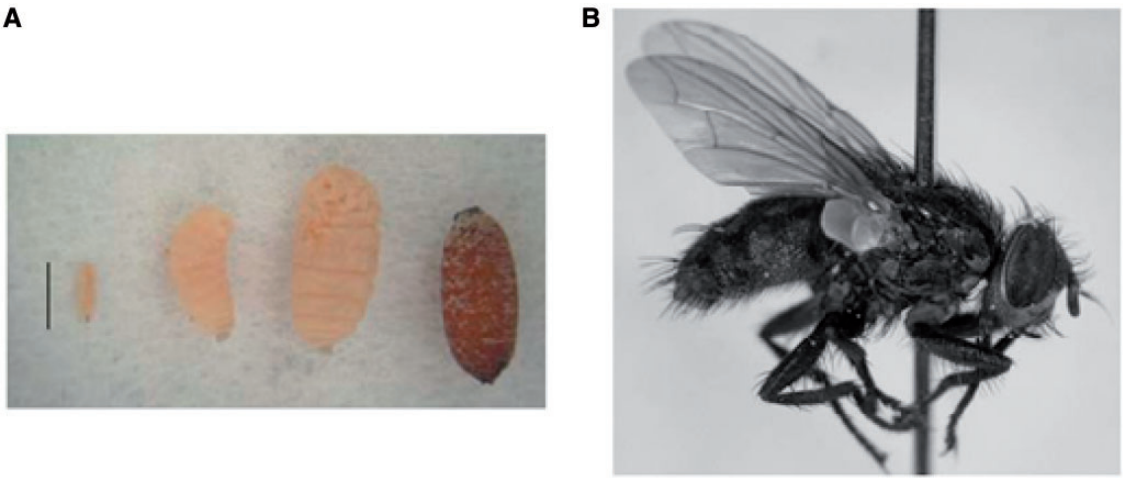

Fig. 1. *Blaesoxipha alcedo* (Sarcophagidae): (A) three larval stages and pupa, and (B) adult. This fly parasitizes *C. c. cyanellus* under natural conditions in the northern part of the state of Veracruz, Mexico. The line in (A) is 1 mm long.

is a natural enemy of *C. c. cyanellus*, and could have a detrimental effect on this beetle species at the population level, and perhaps on other populations of Scarabaeinae. Further studies are required to fully understand the biology of this Sarcophagidae species, and to analyze the long-term effects on the dynamic of *C. c. cyanellus* populations, and those of other dung beetles that are potential hosts of this parasitoid.

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