Biological Studies on Two Sibling Species of *Lixophaga* (Diptera: Tachinidae), Parasites of the New Guinea Sugarcane Weevil, *Rhabdoscelus obscurus* (Boisduval).[1,2]

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The New Guinea sugarcane weevil, *Rhabdoscelus obscurus* (Boisduval), (also known as the sugarcane borer in Hawaiian literature), is the most serious insect pest of sugarcane in the Hawaiian Islands. This weevil, which appears to be native to New Guinea and adjacent islands, is now widely distributed in the tropical western Pacific (Anonymous, 1971). Apparently it reached Hawaii about 1854, possibly in sugarcane from Tahiti. Damage was first observed here at Lahaina, Maui, in 1865 (Muir and Swezey, 1916).

A tachinid parasite, *Lixophaga sphenophori* (Villeneuve), was successfully introduced into Hawaii from New Guinea by Muir in 1910 after a long and arduous search for natural enemies of the weevil in the Orient, Indonesia, and New Guinea (Muir and Swezey, 1916; Pemberton, 1948). The parasites liberated in Hawaiian cane fields all were descended from flies collected by Muir near Port Moresby, at the confluence of the Laloki and Goldie Rivers (Muir, 1910). The establishment of *L. sphenophori* in Hawaii was followed by marked reductions in weevil damage, although minor losses continued to occur on some plantations (Muir and Swezey, 1916). In discussing the status of the pest, Pemberton (1948) stated that the replacement of soft rind cane varieties by medium to hard rind varieties, plus the effect of the New Guinea parasite, "has brought the cane borer problem in the Territory to one of minor importance and damage today is so small as to be hardly measurable." However, during the ensuing 20 years, damage from borers was noted increasingly until on certain plantations, particularly in the windward areas of Kauai, it again became a major economic problem (Experiment Station, HSPA, unpublished). The reasons for the resurgence of borer damage in these areas are not well understood, although it appears that the planting of more susceptible clones, and changes in cultural practices (e.g.: a general lengthening of the period from plant to harvest) may have been responsible. During 1968 a cooperative research pro-

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gram, with the objective of more efficient control of *R. obscurus,* was initiated by the Experiment Station, HSPA; the Entomology Division, Hawaii State Department of Agriculture; and the Entomology Department, University of Hawaii, College of Tropical Agriculture. As a part of this research effort, F.A. Bianchi, entomologist for the Experiment Station, HSPA, was sent to New Guinea to search for additional natural enemies of the borer. One of Bianchi's objectives was to secure populations of *L. sphenophori* from as many different localities and climatic conditions as practicable. It was thought that because the existing Hawaiian population of the parasite was derived from a relatively small number of individuals from a single lowland New Guinea locality, the introduction of new strains of the same species might increase the gene pool of the Hawaiian population through hybridization. Furthermore, it was felt that with the addition of new strains and hybrids, populations might develop which would be more efficient in limiting *R. obscurus* populations over a wider range of cane field environments.

Bianchi sent 16 shipments of *Lixophaga* puparia from Australian New Guinea between April and November, 1968; seven from Wau, four from Popondetta and five from Garaina. These localities are indicated on the map (Fig. 1). *Lixophaga* populations from Wau and Garaina were successfully propagated in the insectary of the Hawaii State Department of Agriculture and several thousands of adult flies from these cultures were liberated in Hawaiian cane fields (Davis and Chong, 1969; Davis, 1971). The Popondetta population was lost before it could be studied or liberated. At the date of this writing (June, 1973) there is no evidence that the Wau-Garaina *Lixophaga* species has become established in Hawaii.

Wau and Garaina are both highland localities and *Lixophaga* from these areas were collected in relatively cool, dry environments. At Wau flies were obtained mostly between 4,000 and 4,500 feet elevation, but some were found as high as 6,000 feet. The Garaina flies were collected at about 2,500 feet (Bianchi, personal communication). Bianchi noted that flies from these highland localities differed slightly from Hawaiian *L. sphenophori* both in appearance and in behavior. However, samples from both Wau and Garaina were submitted to tachinid specialists at the U.S. National Museum and at the British Museum (Natural History) and were determined as *L. sphenophori.*

Two studies pertaining to *Lixophaga* parasites of *R. obscurus* were carried out under the direction of the junior author. One was a field ecology study to determine factors influencing parasite distribution and abundance (Leeper, 1973). The second study, results of which are reported in this paper, centered on breeding experiments to evaluate cross fertility of the available *Lixophaga* populations and interpopulation hybrids, and concomitant laboratory studies on the biologies of these populations.

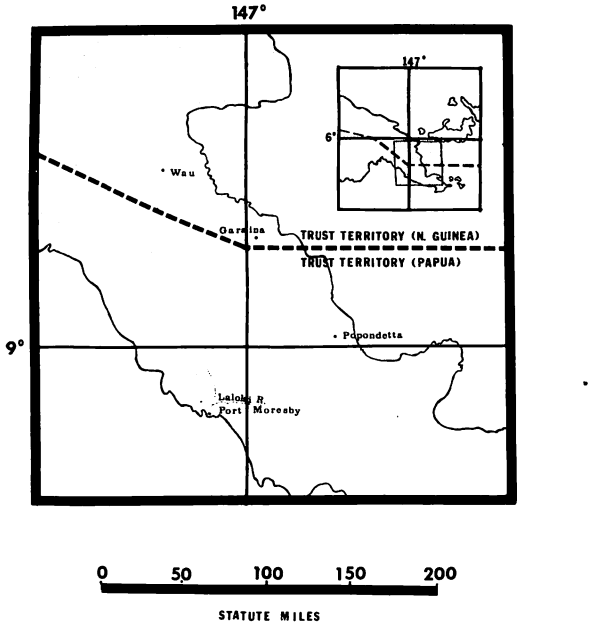

FIG. 1. Map of eastern New Guinea indicating areas where *Lixophaga* populations were obtained.

MATERIALS AND METHODS

The laboratory propagation of *Lixophaga* parasites required a continuous supply of mature *R. obscurus* grubs. These were produced in our laboratory by the method developed by the Entomology Division, Hawaii State Department of Agriculture (Au, 1968).

For hybridization experiments, flies were confined in large cages (71 cm x 53 cm x 46 cm or larger) made of plywood, fine mesh screen and glass. Smaller cages were found to be inadequate as flies confined to them did not mate. Apparently, prenuptial flight, which is a part of the mating behavior in these flies, is inhibited in cages of small volume. Cages were positioned so that they were partially exposed to morning sunlight as our observations indicated that mating usually occurred in sunlight broken by patches of shade, during morning hours. Flies were furnished a food solution, consisting of honey and yeast hydrolysate dissolved in water, which was sprayed on ti *(Cordyline)* leaves at three- or four-day intervals. Water was provided continuously by means of saturated dental wicks. Perforated five dram plastic vials, each containing a mature *R. obscurus* grub in macerated green coconut husk, were suspended in breeding cages for larviposition. These were replaced weekly, and the previously exposed vials were removed to holding cages for adult parasite emergence. Vials were uncapped on the nineteenth

day after initial exposure, and uncapped vials were dampened daily by spraying with atomized tap water to prevent desiccation. Emerged adult *Lixophaga* were removed daily from holding cages, sexed, counted, and either used in hybridization experiments or returned to stock cages. About 30 days after initial exposure, vials were emptied and the fate of the *R. obscurus* grubs determined. Data on percentage of exposed grubs parasitized, number of parasites which emerged from each grub, and pre-emergence parasite mortality were recorded. Stocks of the Wau population and of a Hawaiian population obtained at Ewa Plantation, Oahu, were maintained continuously for 18 months by these means. Our original Garaina stock was lost during a prolonged period of hot weather in September, 1969. After airconditioning our laboratory in June, 1970, we were able to maintain a stock of Garaina flies.

All female flies used in hybridization experiments were dissected and examined soon after death for evidence of embryo development.

Results of Hybridization Tests

Eight pairs of reciprocal crosses, Hawaii ♂♂ x Wau ♀♀ and Hawaii ♀♀ x Wau ♂♂, were attempted. The numbers of flies utilized in each test varied according to the availability of flies, ranging from a low of 4 to a maximum of 95 females in a given cross. The number of males utilized usually was about equal to or slightly greater than the number of females. The average number of flies used in the 16 tests was 39 females and 42 males.

In seven of these crossing attempts no viable progeny resulted. The third test in this series produced viable progeny, but these anomalous results appear to have been due to a laboratory error which resulted in males and females from the same stock cultures being placed together in the hybridization cages. Our suspicion that such an error had been committed was substantiated by morphological examination of the doubtful hybrids and from results of backcrossing attempts. Progeny from the doubtful Hawaii ♀♀ x Wau ♂♂ cross were morphologically like Wau flies and were successfully backcrossed to Wau flies but not to Hawaii flies. The failure of five subsequent attempts to repeat the Wau x Hawaii cross further substantiated our conclusion that the positive results of the third test of this series were due to a laboratory error.

Two pairs of reciprocal crosses of Hawaii x Garaina flies were carried out with negative results. One pair of reciprocal crosses of Wau x Garaina flies yielded F_1 hybrids which were interfertile.

Mating between Hawaii and Wau flies was observed on one occasion, and dead first instar larvae occasionally were present in the oviducts of females used in these crosses. These findings indicate that mating between the two populations sometimes occurred, but that the resulting progeny were inviable. From the results of our hybridization tests we

concluded that the Wau and Garaina populations are conspecific and that these populations represent a distinct, apparently undescribed species of *Lixophaga* which we consider to be a sibling species of *L. sphenophori.*

MORPHOLOGICAL COMPARISON OF LIXOPHAGA SPECIES

In view of the results which we obtained in hybridization tests, detailed examinations of adults and larvae of *L. sphenophori* and the Wau-Garaina sibling species were made in an attempt to find morphological differences. Adult specimens also were submitted to Dr. Roger Crosskey at the British Museum (Natural History), London for his opinion.

Adults of *L. sphenophori* and those of the Wau-Garaina sibling species appear nearly identical. However, in unrubbed specimens there is a slight but consistent difference in the color in the dorsal pollinosity. In Wau-Garaina specimens the pollinosity, particularly that of the scutum and scutellum, has a slight golden yellow tinge, whereas in *L. sphenophori* the pollinosity is more uniformly grayish. These differences are difficult to appreciate unless specimens of the two species are compared directly. Color differences in dorsal pollinosity were noted also by Crosskey (personal communication).

The male genitalia of the two *Lixophaga* species exhibit slight differences at least one of which appears to be statistically significant. A difference in the shape of the hypandria of the two species was first observed by K. Kaneshiro of the University of Hawaii, Department of Entomology (personal communication). In ventral view, the hypandrium of the Wau-Garaina species (Fig. 2C) is relatively shorter and broader than in *L. sphenophori* (Fig. 2D). In flies which we examined, differences in the length/width ratios of the hypandria were statistically significant ($P < 0.05$) (Table I). As the ranges of the length/width ratios of the two species overlap, this character is of little use in the identification of individual specimens. Other minor differences in the male genitalia, such as in the shape and curvature of the cerci (Fig. 2), appear to exist, but have not been compared statistically.

The most useful characters which we were able to find for separating the two *Lixophaga* species were morphological differences in the larvae.[4] Although the first instar larvae of the two species appeared to be identical, second and third instar larvae could be identified by examining the mouth hooks and caudal spiracles.

First instar larvae of both species possess a single simple mouth hook with a blunt, downcurved apex (Fig. 3A). Second and third instar larvae have a pair of mouth hooks. In the second instar, these are elon-

[4]These comparisons were made with Wau and Hawaiian *L. sphenophori* populations as we were unable to maintain the Garaina population in our laboratory during the period when this part of the work was done.

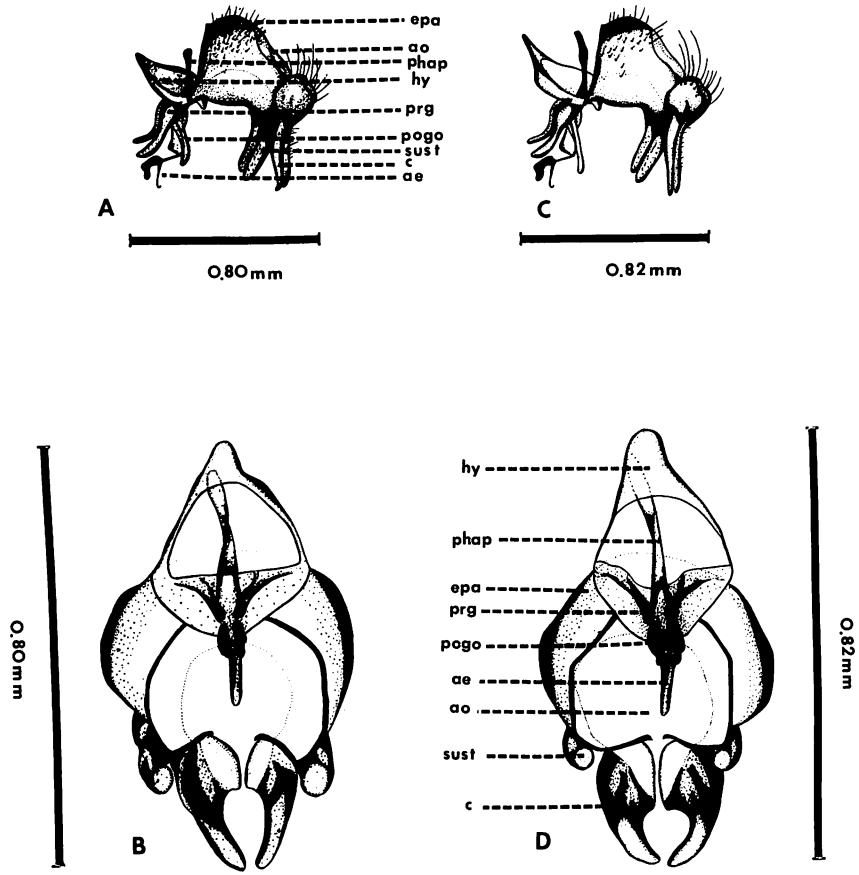

FIG. 2. Male genitalia of *Lixophaga* spp. A, "Wau", lateral aspect. B, "Wau", ventral aspect. C, *L. sphenophori*, lateral aspect. D, *L. sphenophori*, ventral aspect. (ae = aedeagus; ao = anal opening; c = cerus; epa = epandrium; hy = hypandrium; phap = phallophore; pogo = postgonite; prg = praegonite; sust = surstylus).

TABLE 1. *Ratios of length to width measurements of hypandria of "Wau" and "Hawaiian" Flies*

Species	*No.* ♂♂	*Mean* $\frac{length}{width}$	*SD*	*Range of* l/w^a	*Coefficient of variation*
"Wau"	32	1.18	.143	1.00 to 1.67	.1211
"Hawaiian"	60	1.57	.157	1.14 to 1.94	.1001

[a]Length/width.

There is a significant difference at the 5% confidence level for the ratios of length/width measurements of the hypandria of "Wau" and "Hawaiian" flies.

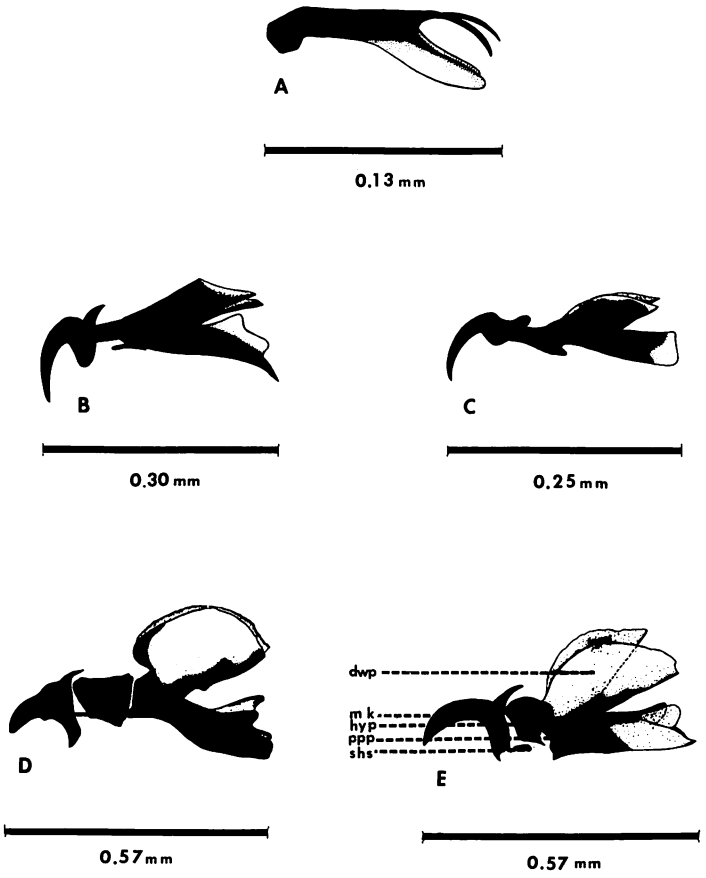

FIG. 3. Mouth hooks and cephalopharangeal skeletons of *Lixophaga* spp. larvae. A, first instar (species indistinguishable). B, second instar "Wau". C, second instar *L. sphenophori*, D, third instar "Wau". E, third instar *L. sphenophori* (dwp = dorsal wing plate; hyp = hypostomium; mk = mouth hook; ppp = posterior pharangeal plate; shs = subhypostomal sclerite).

gated and sharply pointed anteriorly. In Wau larvae the rounded posterior process of the mouth hook is conspicuously larger than in *L. sphenophori* (Fig. 3; B, C). Third instar larvae can be separated readily by differences in the mouth hooks and in the cephalopharangeal skeletons (Fig. 3; D, E). In Wau maggots the anterior portion of each mouth hook is relatively broad (wider than the posterior process), whereas in *L. sphenophori* this part is about the same width or narrower than the posterior process. The hypostomium is approximately quadrangular in lateral aspect in Wau maggots, but in *L. sphenophori* the dorsal margin is rounded and there is a small ventral posterior projection.

The caudal spiracles provide the most easily observable characters for separating mature larvae of the two *Lixophaga* species. Both species have schizotreme stigmata (i.e.: each stigma composed of an oval or circular sclerotized plate with thickened edges which form a distinct ring enclosing the spiracular opening (Fig. 4). Normally there are three slits on each stigma, but occasionally an additional small circular opening, possibly a vestigial fourth slit, is present. In both species each of the three slits has many delicate sclerotic bars extending from the slit wall into the atrium. Each stigma has a small button or scar within the reticulum of the stigmatic chamber.

Differences between mature larvae of the two species were found both in the shapes of the spiracular stigmata in the relative distance between stigmata. In Wau flies the stigmata are relatively elongated and usually somewhat reniform, averaging 0.16 mm wide by 0.25 mm high (Fig. 4A). In *L. sphenophori* larvae stigmata are nearly circular, averaging about 0.19 mm wide (Fig. 4B). In Wau maggots the spiracles are relatively close together, being separated by a distance equal to about half the width of a stigma, while in *L. sphenophori* the interstigmal distance averages about 0.8 the width of a stigma (Table 2).

The number of papillae on the prothoracic spiracles of third instar larvae of the two species were also compared for possible interspecific

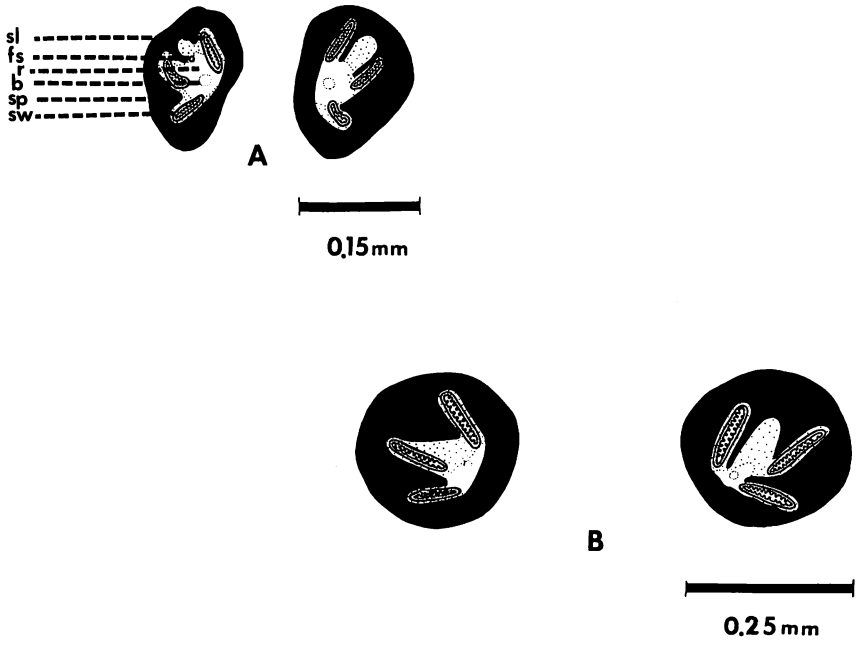

FIG. 4. *Lixophaga* spp.; posterior spiracles of mature larvae. A, "Wau". B, *L. sphenophori*.

TABLE 2. *Comparison of interspiracular distances of posterior spiracles of "Wau" and "Hawaiian" third-instar larvae*

Species	*No. larvae*	*Mean I.S. mm*[a]	*SD*	*Range of I.S. mm*[a]	*Coefficient of variation*
"Wau"	14	.075	.0157	.050 to .100	.209
"Hawaiian"	11	.146	.0323	.083 to .216	.221

[a]Interspiracular distance in mm.

There is a significant difference (P .05) between the interspiracular distances of the posterior spiracles of "Wau" and "Hawaiian" flies.

differences. The number of papillae seems to be positively correlated with maggot size, and, as Wau maggots averaged larger than *L. sphenophori* maggots (due apparently to lower degree of superparasitism in grubs parasitized by Wau flies) Wau maggots had a significantly greater number of papillae. This character does not appear to be of much utility for identification, however.

In addition to the slight morphological differences detailed above, some minor biological differences between the Wau population of the Wau-Garaina species and *L. sphenophori* were noted in our laboratory cultures. For example, the mean number of adult parasites which developed per parasitized host grub was significantly greater in *L. sphenophori* ($\bar{x} = 4.70$) than in the Wau population ($x = 2.87$) (Table 3). This difference also was reflected in the larger average size of the Wau flies produced.

In our laboratory cultures, Wau flies parasitized a significantly higher percentage of the *R. obscurus* grubs exposed to them than did *L. sphenophori*. The overall percentages of parasitism by caged flies was 56.1% for Wau and 49.2% for *L. sphenophori*. The Wau culture yielded an average of 7.89 adult progeny per female fly while *L. sphenophori* yielded 4.44 progeny per female. Since the Wau stock had been cultured under insectary conditions for approximately one year prior to the initia-

TABLE 3. *Comparison of number puparia/parasitized host for beetles exposed to "Wau" and "Hawaiian" Flies*

Species	*No. beetles parasitized*	*Mean no. pup./host*[a]	*SD*	*Range of no. pup./host*[a]	*Coefficient of variation*
"Wau"	399	2.87	1.38	1- 8	.481
"Hawaiian"	393	4.70	3.04	1-15	.647

[a]Puparia/host.

There is a significant difference (P .05) for the number of puparia/parasitized host for "Wau" and "Hawaiian" flies.

tion of the *L. sphenophori* culture, it is possible that the greater parasitism and fecundity exhibited by the former may have been due to the development of a partially cage-adapted race.

Although detailed longevity data were not collected, we found that caged adult flies of both species often lived for 35 days or more, and 30-day-old females were still capable of larviposition. When excessive superparasitization occurred (more than about six or seven flies per host) the resulting flies were undersized and generally died sooner than normal sized flies.

Summary

Cross breeding experiments with *Lixophaga* populations from New Guinea and Hawaii indicated that Hawaiian *L. sphenophori* is reproductively isolated from populations recently imported from Wau and Garaina in eastern New Guinea. *Lixophaga* from these two highland localities were interfertile and appear to represent an undescribed sibling species of *L. sphenophori*. The ancestors of the Hawaiian population of *L. sphenophori* were obtained from a lowland locality near Port Moresby, and the undescribed form possibly represents a highland homologue of lowland *L. sphenophori* in New Guinea.

Minor differences were found in the color of the dorsal pollinosity and the structure of the male genitalia in adults of the two species. Mature larvae were readily separated by morphological differences in the posterior spiracles, mouth hooks and cephalopharangeal skeleton.

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