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BIOSYSTEMATIC STUDIES IN THE *APHYTIS LINGNANENSIS* COMPLEX

YAEL ARGOV[1], EINAT ZCHORI-FEIN[2] AND DAVID ROSEN[2]

[1]*The Israel Cohen Institute for Biological Control, Bet Dagan, Israel*

[2]*The Hebrew University, Faculty of Agriculture, Rehovot, Israel*

ABSTRACT

Aphytis lingnanensis Compere (Hymenoptera: Aphelinidae) is one of the most effective natural enemies of the California red scale, *Aonidiella aurantii* (Maskell) (Coccoidea: Diaspididae) and has been successfully used in the biological control of this important pest. The present study was aimed at establishing the systematic relationships between morphologically identical biparental and uniparental lines of A. *lingnanensis* from different geographical sources. Reciprocal crosses were performed between A, *lingnanensis* from California (the type population) and lines from South Africa, Israel, the Philippines (biparental and uniparental), and a mixture of lines from Texas and Hong Kong. The South African line was also crossed with the Texas-Hong Kong mixture. In order to establish the biosystematic status of the uniparental line, males from California, South Africa, Israel and the Philippines were mated with uniparental females, and female spermathecae were dissected and examined for the presence of sperm. In addition, production of males was induced in the uniparental line by antibiotic treatments, and reciprocal crosses were conducted between that line and the California line. The results showed that A. *lingnanensis* from California was compatible with all biparental lines but the South African one. It was partially compatible with the uniparental lines from the Philippines.

KEYWORDS: Aphelinidae, crosses, hybridization, Hymenoptera, species.

INTRODUCTION

The genus *Aphytis* (Hymenoptera: Aphelinidae) comprises minute, solitary or facultatively gregarious species, ectoparasitic upon armored scale insects (Homoptera: Diaspididae). Due to its great importance in biological control, it has been the subject of many research projects concerning the biological habits as well as the systematic relations among member species (Rosen and DeBach, 1979). In a systematic revision of *Aphytis*, these authors stated that the *lingnanensis* group may contain additional species to the ones already described. The information accumulated in many studies has demonstrated the inadequacy of taxonomic approaches based solely on morphological characters and has stressed the need for a more sophisticated biosystematic approach in order to gain a better understanding of intrageneric systematic relationships (Rao and DeBach, 1969). A good example is the work of Rossler and DeBach (1972), who were able to demonstrate gene flow between arrhenotokous and thelytokous lines of A. *mytilaspidis* (Le Baron).

In the framework of a biological control project directed against armored scale insect pests, several species of *Aphytis* were imported into Israel (Argov and Rossler, 1988). It was soon

realized that morphological criteria were insufficient for identification of *Aphytis* species in the *lingnanensis* complex. The goal of the present study was to establish the biosystematic relations between these *Aphytis* lines. Two criteria were used in order to measure reproductive isolation: (1) fertilization: because *Aphytis* species are haplodiploid, production of female offspring by mated females in biparental lines indicates that fertilization has occurred, and (2) insemination: spermathecae of mated females were dissected in order to test for sperm transfer. In the study we took advantage of our ability to induce production of males in a uniparental line (Zchori-Fein et al., 1994), thereby overcoming the obstacle usually presented by parthenogenetic lines.

MATERIAL AND METHODS

Insects. *Aphytis* lines from several geographical origins were imported into Israel and identified as *A. lingnanensis* Compere on the basis of their morphological characters. Their origin, year of importation, the abbreviation used and original host are summarized in Table 1. *A. lingnanensis* was described in California from material of Chinese origin (Compere, 1955), and the California (CA) line was therefore used as a reference for all other lines. The compatibility of the lines received from Texas (TX) and Hong Kong (HK) was tested upon their arrival; they were found to be conspecific and their cultures were therefore united. The *Aphytis* lines imported from South Africa in 1987 and 1989 (SA87 and SA89) were cross-mated and found to be the same species. A uniparental (PhUP) and a biparental (PhBP) line were shipped from the Philippines, and an azinphosmethyl-resistant strain was collected in a citron orchard in Israel (IS) (Havron et al., 1991).

TABLE 1
Lines of *Aphytis*, identified as *A. lingnanensis*, crossed in Israel

Country of origin	Year of importation	Abbreviation	Host	Mode of reproduction
California	1988	CA	*Aspidiotus nerii*	biparental
Texas	1976	TX	Diaspididae[2]	biparental
Hong Kong	1976	HK	Diaspididae[2]	biparental
South Africa	1987	SA87	*Aonidiella aurantii*	biparental
South Africa	1989	SA89	*Aonidiella aurantii*	biparental
Philippines	1988	PhBP	*Chrysomphalus aonidum*	biparental
Philippines	1987	PhUP	*Aonidiella* spp.	uniparental
Israel	1986[1]	IS	*Aonidiella aurantii*	biparental

[1]Azinophosmethyl-resistant strain, collected from a citron orchard.
[2]Apparently from a contaminant in a *Parlatoria pergandii* shipment.

Experimental procedure. Unless otherwise stated, pupae were individually isolated in a glass vial or a gelatin capsule and the emergent virgin adults were used within 24 h. All experiments were conducted under 25 ± 2°C and 60% RH. In order to induce production of males in the uniparental line from the Philippines, females were fed with 50 mg/ml rifampicin in honey (Zchori-Fein et al., 1994). The biosystematic relationships between the various *Aphytis*

lines was tested on cross-mated females in two ways: (1) the percentage of the female offspring produced, and (2) the presence of sperm in the spermathecae.

Offspring produced by cross-mated females. In order to test whether fertilization occurs between the various lines of *Aphytis*, one male and one female from different lines were paired and watched copulating. After 2 hours, the pair was placed for oviposition in a small plastic cylinder, attached to a squash infested with an excess number of suitable armored scale hosts. The cylinder was then covered with a piece of cloth and the squash was kept in a rearing chamber until adult progeny emerged, which were then counted and sexed. The hosts used and the number of replicates are listed in Table 2.

Occurrence of sperm in spermathecae. The presence of sperm in the spermathecae was observed in females cross-mated under two different conditions: (a) individual couples: 9–18 heterospecific couples were placed individually in glass vials with honey for 1–72 hours (Table 3); and (b) large groups: a varying number of males and heterospecific females, 0–72 hours old, were placed together in a test tube for different periods of time (see Table 4 for details). The females were then dissected in a drop of physiological solution (0.9% NaCl) on a microscope slide. The spermatheca was carefully removed, covered with a coverslip and immediately examined under a compound microscope for the presence of active sperm.

RESULTS

Offspring produced by mated females. Production of female offspring was used to determine the biosystematic relationships between the various lines. The percentage of female progeny found in reciprocal crosses between the CA and the TX-HK line was around 60%. Only males were found when the TX-HK line was crossed with the SA87 line (Table 2). The same result was obtained when the SA87 line was crossed with the CA line. However, at least one female

TABLE 2
Sex ratio of offspring resulting from crosses between various *Aphytis* lines identified as *A. lingnanensis*

Crosses (females × males)	n[1]	No. of couples with female offspring	No. of female offspring	No. of male offspring	% females	Host
CA × CA	10 (9)	9	28	15	65	*Aspidiotus nerii*
CA × TH	21 (17)	13	54	61	47	*Aspidiotus nerii*
TH × CA	20 (17)	16	85	44	66	*Aspidiotus nerii*
TH × SA87	60 (49)	0	0	43	0	*Aonidiella aurantii*
SA87 × TH	60 (35)	0	0	73	0	*Aonidiella aurantii*
CA × SA87	22 (19)	0	0	111	0	*Aspidiotus nerii*
SA87 × CA	22 (20)	0	0	79	0	*Aspidiotus nerii*
CA × SA89	12 (12)	1	5	188	2.5	*Aspidiotus nerii*
SA89 × CA	5 (5)	1	1	61	1.6	*Aspidiotus nerii*
SA89 × CA	10 (10)	1	1	130	0.7	*Aonidiella aurantii*

[1]Number of couples tested. The number of couples that actually produced offspring is given in parentheses.

was produced in reciprocal crosses between the CA line and the line imported from South Africa in 1989.

Occurrence of sperm in the spermathecae. In order to establish the biosystematic relationships between the uniparental line and other *Aphytis* lines, the spermathecae of cross-mated females were dissected. The results show that sperm was transferred both ways between the biparental line from the Philippines and the California *A. lingnanensis* (Table 3). No sperm was found in the spermathecae of uniparental females mated with males from all lines other than their own (Table 3). Correspondingly, over 95% of males from the uniparental line were incapable of inseminating females from the CA line, although they were fully capable of inseminating their own females (Table 4).

A summary of the biosystematic relationships among the *Aphytis* lines imported into Israel and identified as *A. lingnanensis* is presented in Fig. 1.

TABLE 3
Presence of sperm in spermathecae of cross-mated *Aphytis* female
(one female mated with one male)

Crosses (females × males)	Time together (h)	n[1]	Spermathecae with sperm	
			No.	%
PhBP × CA	4–28	10	7	70
CA × PhBP	26	10	5	50
PhUP × PhBP	1–18	18	0	0
PhUP × CA	2–72	9	0	0
PhUP × SA	19–23	12	0	0
PhUP × IS	2–18	12	0	0

[1]Number of couples tested.

TABLE 4
Presence of sperm in the spermathecae of cross-mated *Aphytis* females
(a group of several males and females)

Crosses (females × males)	Time together (h)	n[1]	No. of wasps		Spermathecae without sperm	Spermathecae with sperm	
			Females	Males		No.	%[2]
PhUP × CA	2–72	10	126	94	66	0	0
PhUP × SA	2–24	4	94	61	58	0	0
PhUP × PhBP	3–24	3	33	24	33	0	0
PhUP × PhUP	20–24	3	39	26	13	15	53.6
CA × PhUP	20–24	4	30	28	20	1	4.8

[1]Number of groups tested.
[2]Percentage of females dissected.

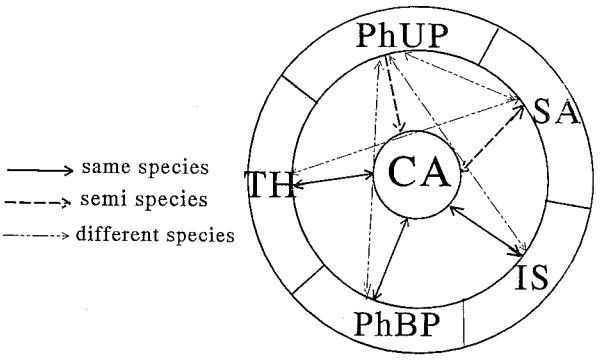

Fig. 1. Crossing relations among lines of *Aphytis* identified as *A. lingnanensis*, tested in Israel. CA = Riverside California; IS = Israel; PhBP = Philippines biparental; PhUP = Philippines uniparental; SA = South Africa; TH = Texas–Hong Kong mixture.

DISCUSSION

The term "semi-species" refers to conspecific populations that are partially reproductively isolated and can be distinguished and evaluated only by crossing tests (Mayr, 1963; DeBach, 1969).

In arrhenotokous species of *Aphytis*, females usually constitute 60–70% of the population (Rosen and DeBach, 1979). The data obtained in the present study show that the *Aphytis* lines from Texas, Hong Kong and the biparental line from the Philippines are all compatible with the California line, and therefore can all be determined as *A. lingnanensis*. The results obtained from crosses between the biparental line from the Philippines and the California line are in agreement with the findings of A. Havron (personal communication), who obtained over 44% females in reciprocal crosses between these lines. The original source of both the California and the Texas line was a shipment imported into California from South China in 1947 (DeBach, 1960).

Although *A. lingnanensis* had been imported into Israel in 1960 (Rosen, 1967), this species was not found in Israel in a survey carried out in the mid-1970s (Kamburov, 1974). A second attempt to establish *A. lingnanensis* in Israel was made by the Israel Cohen Institute of Biological Control in 1977. At that time, wasps from the mixture of Texas and Hong Kong lines were released (Anonymous, 1979). In 1985, an azinphosmethyl-resistant line of *A. lingnanensis* was recovered from a citron orchard in Israel (Havron et al., 1991). Reciprocal crosses between that resistant strain and the California line yielded about 60% females (Havron et al., 1991). It is therefore reasonable to assume that the resistant strain has been derived from wasps from previous releases, and can also be determined as *A. lingnanensis*.

The results also show that males from all tested lines were unable to inseminate females from the uniparental line of *Aphytis* from the Philippines (Tables 3 and 4). Although males of that line, induced by antibiotic treatments, are capable of inseminating their own females, sperm was not transferred from them to any other line, except for one female from the California line (Tables 3 and 4). These results indicate that this uniparental line is on its way to becoming completely reproductively isolated from *A. lingnanensis* and can be termed a semi-species in relation to the former.

The data show that the South African line imported in 1987 is reproductively isolated from

the *A. lingnanensis* complex (Table 2). In contrast, few females were found in reciprocal crosses between the South African line imported in 1989 and the California line. It is possible that early death of alien sperm in the spermathecae of females, or hybrid inviability, or both, may be the cause of that low reproductive success (Rao and DeBach, 1969). The pattern observed fits the definition of semi-species (DeBach, 1969).

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