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BIOSYSTEMATIC STATUS OF THREE ALLOPATRIC POPULATIONS OF *APHYTIS MACULICORNIS* [*HYM.: APHELINIDAE*] (1)

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Biological differences among 3 morphologically identical populations of *Aphytis* which would key down to *A. maculicornis* (MASI) are presented through studies conducted on their reproductive isolation, host preferences and adult survivorships at different temperatures. These results supplement results on experimental hydridization and provide ample evidence that from a practical biological control stand-point as well as from basic biosystematics, these 3 populations are valid species, distinct from each other. The significance of such sibling species in relation to biological control is discussed.

The aphelinid species *Aphytis maculicornis* (MASI) includes several populations of different geographic origin which are morphologically identical, yet exhibit remarkable distinctness from one another in their biological attributes (HAFEZ & DOUTT, 1954; KHASIMUDDIN & DEBACH, 1976). The latter authors have discussed the significance of such biological differences in relation to biological control, with respect to 3 populations originating from Persia, Pakistan and California, and have suggested that these 3 populations may be distinct species with respect to one another. The present paper deals with additional evidence for the same conclusion based on studies conducted on their reproductive isolation, host preferences and other ecological factors.

It is essential that the past histories and origins of the 3 populations we studied be clarified inasmuch as HAFEZ & DOUTT (1954) in their work on biological evidence of sibling species in *A. maculicornis* categorized 4 distinct forms as Persian, Indian, Spanish and a supposedly indigenous California strain.

Our "Persia" population is the same as the Persian strain of HAFEZ & DOUTT (1954). Ours originated from stock cultures maintained at the insectary at Albany, University of California, Berkeley. At Albany the culture was started from San Joaquin Valley field-collected parasites attacking olive scale, *Parlatoria oleae* (COLVEE), which were descendants of the Persian *A. maculicornis* introduced from Iran during 1951. This was the only one that became established on this scale (HUFFAKER *et al.*, 1962), and furthermore, *A. maculicornis* did not occur in California prior to 1949 (DOUTT, 1954).

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The California strain referred to by HAFEZ & DOUTT (1954) is not the same as the "California" population of the present study. The former was reported to have been collected in the San Joaquin Valley in Central California on *Parlatoria oleae* and was also reported as being completely thelytokous. The latter, "California" population, by contrast, was collected at Escondido in southern California from *P. pergandii* and is distinctly arrhenotokous. It is hardly conceivable that the San Joaquin Valley strain could have evolved into the population found at Escondido by crossing the mountain barriers, reaching Escondido, and above all, changing its mode of reproduction in only 15 to 20 years. While its presence in Escondido remains an unanswered question, the information at hand clearly indicates that our "California" population is definitely different from the California strain referred to by HAFEZ & DOUTT (1954).

Our "Pakistan" population was received at the Quarantine Insectary of the Division of Biological Control, University of California, Riverside, in 1964. This form was collected from San Jose scale, *Quadraspidiotus perniciosus*. Pakistan was considered by HAFEZ & DOUTT (1954) as a part of the range for their Indian strain, but their form was collected from olive scale, *P. oleae*. It is not known whether there exists one or more forms of *A. maculicornis* in that general region. However, in view of these different host records, our "Pakistan" population is possibly not the same as the Indian strain of *A. maculicornis* of the above mentioned authors. Further studies would be necessary with these 2 populations to establish their exact identities, but the Indian strain of HAFEZ & DOUTT has not been available to us.

STUDIES ON REPRODUCTIVE ISOLATION

These 3 populations do not interbreed to any extent under normal conditions, but do so to a limited degree under manipulated laboratory conditions using sex pheromone mating inducers (KHASIMUDDIN & DEBACH, 1976). It was felt desirable to evaluate more precisely the degree of reproductive isolation existing between them in order to arrive at more definite conclusions regarding their biosystematics. RAO & DEBACH (1969a) have utilized so-called "multiple-choice" experiments in order to determine the degree of reproductive isolation within members of certain species groups of *Aphytis*.

In the present study, a series of "multiple-choice" experiments were conducted to determine the degree of reproductive isolation that exists between the 3 populations of *A. maculicornis*. A "multiple-choice" experiment conducted for any 2 populations is one in which males of one population are given a choice of mating between homogamic and heterogamic females. The proportion of females of each population thus inseminated is then determined by dissecting the females and examining their spermathecae for the presence of sperm.

In such experiments, since males of one population are given a choice of mating with females of two, it might be mistakenly assumed that males make the ultimate choice in consumation of mating. However, (KHASIMUDDIN & DEBACH, 1975), it is the female that makes the final choice of either accepting or rejecting males of a particular population for mating. In other words, these experiments determine the degree to which females of either of the 2 populations reject the males (RAO & DEBACH, 1969). If equal proportions of homogamic and heterogamic females are inseminated, random mating is indicated which in turn denotes little or no reproductive isolation between the 2 populations.

If, on the other hand, unequal proportions of homogamic and heterogamic females are inseminated, mating is considered to be preferential, which in turn indicates the presence of reproductive isolation (either positive or negative) between them.

MATERIALS AND METHODS

Virgin females and newly emerged males, not more than 24 hours old, were utilized in all the tests. Ten virgin females of each of 2 populations, for example "Pakistan" and "California" (total of 20 females), were anesthetized using CO_2 and transferred into an 8 dram vial supplied with honey streaks for food. Ten newly emerged males of one of the populations, for example "Pakistan", were then anesthetized and transferred into this vial containing the females. All tests conducted in this study were at ca. 27°C and 50 % R.H.

As the populations under study were morphologically identical, distinguishing the females after they have been mixed would be impossible. This difficulty was overcome by pre-feeding the females of one population with honey that was colored by means of a harmless food color (Schilling, McCormick & Co., Inc.). Such females showed the color through their almost transparent abdominal integument. Only females that clearly showed the color were selected for tests. This technique was developed by RAO & DEBACH (1969a) for similar studies with certain species and forms of *Aphytis*.

Each vial containing 20 females and 10 males formed one replicate. Five replicates were run, so in all, 50 females of each population were used. The entire series of experiments was reciprocated using males of the other population. A mating period of 24 hours was allowed. After this period the test individuals in each vial were anesthetized, and the females were separated and labelled accordingly. The males from each vial were destroyed.

The separated females were then, while still alive, dissected in normal saline solution under a phase microscope (100 × 200× and 430×), and their spermathecal capsules were examined for the presence of sperm. This was accomplished by carefully pulling out the ovipositor with a pair of extra-fine laboratory tweezers (# 000), while holding the body of the female with a blunt, smooth needle. This extracted the genitalia together with the brownish, kidney-shaped spermathecal capsules. The presence of sperm in the spermathecae was ascertained by their wriggling and undulating movements within the spermathecal capsules. Care was taken to complete the observation within a few seconds of excising the genitalia, so that the sperm could be still alive and moving when observed, although these sperms can live for 10 to 12 minutes after dissection. A similar technique has long been used by various workers to study reproductive isolation in Drosophila (STALKER, 1942; DOBZHANSKY & MAYR, 1944; DOBZHANSKY & STREISINGER, 1944).

RESULTS AND DISCUSSION

Table 1 shows the frequency totals from 5 replicates of homogamic and heterogamic females inseminated under each "multiple-choice" experiment. It is apparent that homogamic insemination is a rule rather than an exception among the 3 populations of *A. maculicornis* (ranging from 84 % to 96 %) and that heterogamic inseminations are rare (ranging from 0 % to 6 %).

Using data from table 1, the coefficients of isolation ($K_{1,2}$ and $K_{2,1}$) and the coefficients of joint isolation (K_1 and K_2) were calculated according to the formulae developed by LEVENE (1949). LEVENE (1949) states that the coefficient of isolation is an improvement over the CHARLES STALKER "Isolation Index" (STALKER, 1942), being independent of the length of time allowed for mating. The calculated values for the coefficients of isolation are given in table 2 with corresponding standard errors also calculated by formulae developed by LEVENE (1949). Table 2 also gives the χ^2 values for the observed differences between homogamic and heterogamic matings. LEVENE (1949) further states that "the coefficient of joint-isolation measures the true degree of reproductive isolation

between the 2 strains under the (artificial) conditions of the experiment, while the "coefficients of excess insemination" ($m_{1,2}$) can serve as a measure of the extent to which the gene flow between the 2 strains is in one direction only. " A value of 1.00 for the coefficient of isolation indicates complete positive isolation; that of 0.00 indicates no isolation. Increasing values from 0.0 to 1.00 are indicative of the degree of reproductive isolation existing between the 2 strains in question. Similarly, values ranging from 0.00 to —1.00 indicate the degree of negative isolation. The last 2 columns give the calculated values for the coefficient of excess insemination and coefficient of joint isolation.

TABLE 1

"Multiple-choice" experiments: Frequencies of homogamic and heterogamic inseminations by males of one population in the joint presence of honogamic and heterogamic females

Crosses ♀♀	♂♂	Homogamic inseminations n	s	%	Heterogamic inseminations n	s	%
Persia and Pakistan	Pakistan	50	42	84.0	50	0	0.0
Pakistan and Persia	Persia	50	46	92.0	50	3	6.0
California and Pakistan	Pakistan	50	43	86.0	50	1	2.0
Pakistan and California	California	50	46	92.0	50	1	2.0
California and Persia	Persia	50	45	90.0	50	0	0.0
Persia and California	California	50	42	84.0	50	1	2.0

n = number of females used; s = number of females inseminated;
% = percentage of females inseminated.

Computed coefficients of isolation (table 2) range from 0.8936 to 1.00, indicating the predominance of preferential matings over random matings between any 2 forms at a time. The values for the coefficients of isolation thus indicate a high degree of reproductive isolation among the 3 populations. These are further confirmed by χ^2 values in table 2, ranging from 65.2794 to 78.2222, which denote a very highly significant difference ($p < 1.0\,\%$) between homogamic and heterogamic matings.

TABLE 2

Coefficients of isolation, joint-isolation and excess insemination calculated on the basis of "multiple-choice" experiments

Crosses ♀♀	♂♂	Coef. of isolation $K_{1,2}$ & $K_{2,1}$	Standard errors $K_{1,2}$ & $K_{2,1}$	χ^2	Coef. of joint isolation ($K_{1,2}$)	Coef. of excess insemination ($m_{1,2}$)
Persia and Pakistan	Pakistan	1.0000	0.0000	69.0065	0.9756	0.0244
Pakistan and Persia	Persia	0.9512	0.0225	70.5882		
California and Pakistan	Pakistan	0.9796	0.0094	68.2224	0.9366	0.0430
Pakistan and California	California	0.8936	0.0486	77.7197		
California and Persia	Persia	1.0000	0.0000	78.2222	0.9891	0.0109
Persia and California	California	0.9782	0.0102	65.2794		

As stated earlier, the true measure of reproductive isolation, according to LEVENE (1949), is the coefficient of joint-isolation. Figures for I is in table 2 range from 0.9366 to 0.9891. These values indicate a very high degree of reproductive isolation between any 2 populations of *A. maculicornis*. Therefore, the 3 populations of *A. maculicornis* are nearly completely reproductively isolated from one another. The values for coefficients of excess insemination for these forms (0.0109 to 0.0430) also indicate the same trend.

STUDIES ON HOST PREFERENCE

The host range of parasitic Hymenoptera is of practical importance to workers in biological control. It becomes even more significant when one is dealing with morphologically identical populations as in the present case, and may form an important ground of distinction between them.

The 3 populations under study were tested for their host preferences in order to discern differences, if any.

MATERIALS AND METHODS

There were 2 types of experiments designed to study host preferences. In the 1st type a simultaneous "multiple-choice" of 6 differents host scales was provided to the test females, while the 2nd type was essentially a "no-choice" experiment, where only a single host scale species was provided for the test females. The general conditions and procedures for recording progeny from the parasitized host scales were the same for both types of experiments. The 6 host scales utilized in the experiments were :

Cactus scale	*Diaspis echinocacti* (BOUCHE)
Latania scale	*Hemiberlesia lataniae* (SIGNORET)
Oleander scale	*Aspidiotus nerii* (BOUCHE)
Red scale	*Aonidiella aurantii* (MASKELL)
Yellow scale	*Aonidiella citrina* (COQUILLETT)
Purple scale	*Lepidosaphes beckii* (NEWMAN)

In the text that follows, the common names of these scales will be used.

Individual pupae from each population were isolated in the green-eyed (ultimate pupal) stage and held for emergence at 27°C and 50 % R.H. Soon after emergence, matings were set up in groups of 5 females and 5 males. The 5 mated females were transferred to a battery jar in which fresh host scale material from the 6 different hosts was provided in sufficient quantities. This usually consisted of 4 pairs of lemons, each pair bearing red scale, yellow scale, purple scale and oleander scale, respectively, 2 potato tubers bearing latania scale and 3 or 4 cactus pads bearing cactus scale. Honey was provided in thin streaks on the inside of the jars as food for the test females and the jars were covered with a cloth held tight by means of a rubber band. There were 5 such jars set up for each population, making the total number of test females 25.

The females were allowed to oviposit for a period of 10 days initially after which they were anesthetized and transferred to fresh scale material in fresh jars for further oviposition. It was noted that oviposition seldom occurred on the second set of scale material because the fecundity of aged females declines greatly. The host material subjected to oviposition was separated and held for adult emergence in one pint mason jars. Emergence was checked every day after the 14th day from the start until there was no more emergence after 23 to 28 days. All emerging progeny was recorded for each scale species.

For "no-choice" experiments the 5 mated females were given enough host material of a single host scale and separate experiments were set up with each of the 6 host scale species.

RESULTS AND DISCUSSION

Results from "multiple-choice" and "no-choice" experiments are presented separately for each of the populations. Table 3 summarizes results of the "multiple-choice" and "no-choice" experiments for the 5 replicates for the "Pakistan" population.

TABLE 3

Progeny production of the "Pakistan" population on different host scale species in "multiple-choice" and "no-choice" experiments.

Scale species*	Replication no. 1	2	3	4	5	Mean	S. D.	Coef. of variation
				"Multiple-choice"				
Cactus	161	146	133	143	151	146.8	10.3	0.07
Latania	13	10	8	0	0	6.2	2.5	0.24
Total	174	156	141	143	151			
				"No-choice"				
Cactus	181	173	169	163	168	170.8	6.70	0.03
Latania	46	53	49	58	41	47.4	8.01	0.16

* No progeny production occurred in tests using oleander, red, yellow or purple scales.

S. D. = Standard deviation.

No parasitization occurred on oleander, red, yellow, or purple scales, either in the "multiple-choice" or "no-choice" experiments. Progeny production on cactus scale was found to be significantly more than on latania scale in both the types of experiments. When given a choice, this form prefers cactus scale with very few progeny being produced on latania scale ($\bar{x} = 6.2$ for 5 females). However significantly more progeny was produced on latania scale ($\bar{x} = 47.4$ for 5 females) when the ovipositing females had no choice of other host scale species. Therefore, although cactus scale remains most preferred, this population apparently can maintain itself on latania scale.

Table 4 gives the progeny production of the "Persia" population on different host scale species under "multiple-choice" and "no-choice" experiments. No progeny whatsoever was produced on red, yellow, and purple scales. When given a choice of hosts simultaneously, this population produced the most progeny on cactus scale. This progeny was significantly more than that produced on latania or oleander scales. The production of progeny on oleander scale was a feature unique to this population. This was further exemplified in the "no-choice" experiments where the mean progeny per replicate on oleander scale was 145.6 as against 75.0 in the "multiple-choice" experiments. A similar trend for increased mean progeny under "no-choice" was observed for cactus scale and latania scale, but the most important fact here seems to be the high preference for oleander scale in addition to cactus scale and latania scale.

Table 5 summarizes results of progeny production by the "California" population on the 6 different scale species, under "multiple-choice" and "no-choice" experiments respectively.

TABLE 4

Progeny production of the "Persia" population on different host scale species under "multiple-choice" and "no-choice" experiments.

Scale species*	Replication no. 1	2	3	4	5	Mean	S. D.	Coef. of variation
				"Multiple-choice"				
Cactus	97	103	86	89	81	93.2	6.79	0.07
Latania	11	21	19	24	27	20.4	6.06	0.29
Oleander	72	66	89	75	73	75.0	8.51	0.11
Total	180	189	194	188	191			
				"No-choice"				
Cactus	176	192	203	187	187	189.0	9.77	0.05
Latania	43	56	58	61	54	54.6	6.80	0.12
Oleander	151	146	139	143	149	145.6	4.77	0.03

* No progeny production occurred in tests using red, yellow or purple scales.

S. D. = Standard deviation.

TABLE 5

Progeny production of the "California" population on different host scale species under "multiple-choice" and "no-choice" experiments.

Scale species*	Replication no. 1	2	3	4	5	Mean	S. D.	Coef. of variation
				"Multiple-Choice"				
Cactus	168	153	172	181	164	167.6	10.31	0.06
Latania	20	0	0	6	9	7.0	7.37	0.63
Total	188	153	172	187	173			
				"No-choice"				
Cactus	183	171	169	173	167	172.6	6.22	0.03
Latania	33	41	29	37	21	32.2	7.69	0.23

* No progeny production occurred in tests using oleander, red, yellow or purple scales.

S. D. = Standard deviation.

No progeny whatsoever was produced on oleander, red, yellow and purple scales. The preference for cactus scale was significantly greater than for latania. Progeny production on latania scale in the "multiple-choice" experiments shows that very few progeny will occur on this scale, although production was increased somewhat under "no-choice" conditions. Obviously, the only highly preferred host scale for this population is the cactus scale.

Consideration of the host preferences of the 3 forms reveals that the "Persia" population is distinct in that it readily parasitizes oleander scale whereas the others do not at all. All strongly prefer cactus scale to latania scale, but again "Persia" appears to differ from the others in having a somewhat better acceptance of latania scale. The "Pakistan" and "California" forms do not differ much, if any, in their relative choice of, and progeny production on, cactus scale as compared to latania scale. Thus, this

information does not materially help to distinguish between these 2 forms. The possibility exists, however, that had a wider range of host species been tested, marked differences might have appeared.

STUDIES ON LONGEVITY OF ADULTS AT DIFFERENT TEMPERATURES

Differences in adult longevity of morphologically indistinguishable populations of parasitic Hymenoptera can provide additional information regarding their biosystematic relationships. HAFEZ & DOUTT (1954) presented biological evidence of sibling species in *A. maculicornis* and one of the biological attributes of differences was adult longevity.

MATERIALS AND METHODS

During the present studies with these arrhenotokous populations, it was observed that males are relatively short lived. Females survive for longer periods of time, particularly if prevented from ovipositing. For this reason, only females were utilized for experiments on survivorship of adults.

Experiments were conducted under 2 different constant temperatures, ca. 27°C and ca. 32°C, in temperature controlled cabinets maintained at ca. 50 % R.H. One hundred newly emerged females of each of the populations were set-up in 5 3-dram vials, 20 females per vial for each temperature. Each vial was provided with a ventilated cork. Fine streaks of honey were provided inside each vial, care being taken to keep these honey streaks very fine in order to prevent any test individuals from getting trapped in the honey. This honey was replenished whenever necessary. Mortality was recorded at 24 hour intervals.

RESULTS AND DISCUSSION

Results on adult survivorship at 27°C and 32°C are presented in table 6. Survivorship curves were prepared from this data and are presented in figs. 1 and 2 for 27°C and 32°C respectively.

From the table 6 data it was calculated that at 27°C the number of days required for 50 % mortality is as follows: "Persia", 23-24; "Pakistan", 19-20; "California", 13-14. At 32°C the days to 50 % mortality were "Pakistan", 13-14; "Persia", 10-12; "California" 8-9. Thus the 3 populations are biologically distinct in their response to temperature. The "California" population survived poorest at both temperatures, the "Persia" population survived best at 27°C, whereas perhaps the "Pakistan" population survived slightly better at 32°C. The survivorship curves (figs. 1 & 2) show that such differences hold true for the entire period of longevity, especially at 27°C. All in all these tests indicate marked biological differences between the 3 forms, especially the "Pakistan" and "California" forms that did not show appreciable differences in the host preference tests.

DISCUSSION AND SUMMARY

The evidence presented shows that the 3 populations studied are for all practical purposes completely reproductively isolated and have different biological characteristics as measured by host preferences and longevity. Their reproductive isolation is ethological, the sex pheromones playing a major role in maintaining such isolation (KHASIMUDDIN & DEBACH, 1975). Gene exchange, though artificially possible in the laboratory

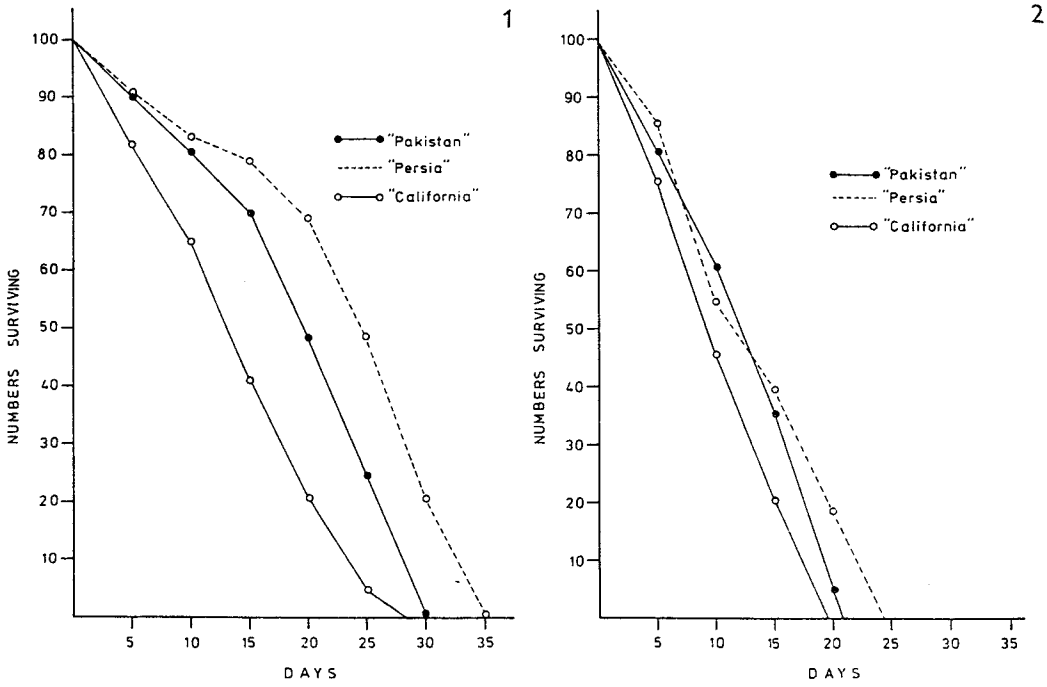

FIG. 1. Survivorship of females of the 3 populations of *A. maculicornis* at ca. 27°C.
FIG. 2. Survivorship of females of the 3 populations of *A. maculicornis* at ca. 32°C.

and with no apparent deleterious after effects (KHASIMUDDIN & DEBACH, 1976), is effectively prevented. In view of the above, the 3 populations of *Aphytis* are considered valid species with respect to one another.

Host preference studies revealed significant differences among the 3 siblings. Cactus scale was the host most preferred by all the 3. According to the mean progeny produced on this scale, the 3 siblings can be ranked as "Persia" (189.0) > "California" (172.6) > "Pakistan" (170.8). Ranking according to the progeny production on latania scale gives "Persia" (54.4) > "Pakistan" (47.4) > "California" (32.2). This means that the 3 siblings can parasitize cactus scale and produce abundant progeny. On latania scale, however, their mean progeny production is limited, the "California" sibling being barely able to survive, with the other 2 producing somewhat better numbers of progeny. Most important, the "Persia" sibling produced progeny on oleander scale of a fairly normal mean value, whereas absolutely no progeny was produced on this scale by either of the other 2 siblings. This property of the "Persia" sibling is again a highly meaningful biological difference of this sibling from the other 2.

The adult survivorships at 27°C and 32°C (figs. 1 & 2) and the number of days required for 50 % mortality were also found to be different for these 3 siblings. The "Persia" sibling was the better survivor at 27°C while "Pakistan" perhaps survived better at 32°C; the "California" sibling exhibited inferior survival at both temperatures. This again points out the distinctness of the 3 siblings with respect to yet another of their biological attributes.

TABLE 6

Survivorship of adult females of the three populations of A. maculicornis, *at two different temperatures* (27°C *and* 32°C)

	27°C						32°C					
	«Pakistan»		«Persia»		«California»		«Pakistan»		«Persia»		«California»	
Day	Nx 1	Lx	Nx 1	Lx	Nx 1	Lx	Nx 1	Lx	Nx 1	Lx	Nx 1	Lx
0	100	1.0	100	1.0	100	1.0	100	1.0	100	1.0	100	1.0
1	99	0.99	97	0.97	89	0.89	94	0.94	90	0.09	90	0.90
2	92	0.92	95	0.95	86	0.86	89	0.89	90	0.90	90	0.90
3	91	0.91	95	0.95	85	0.85	87	0.87	89	0.89	83	0.83
4	91	0.91	93	0.93	84	0.84	86	0.86	89	0.89	81	0.81
5	90	0.90	92	0.92	84	0.84	81	0.81	86	0.86	76	0.76
6	90	0.90	92	0.92	83	0.83	75	0.75	84	0.84	68	0.68
7	86	0.86	91	0.91	80	0.80	73	0.73	72	0.72	56	0.56
8	86	0.86	90	0.90	76	0.76	69	0.69	66	0.66	51	0.51
9	85	0.85	89	0.89	71	0.71	69	0.69	61	0.61	49	0.49
10	81	0.81	86	0.86	65	0.65	62	0.62	54	0.54	46	0.46
11	81	0.81	86	0.86	59	0.59	61	0.61	50	0.50	38	0.38
12	79	0.79	85	0.85	55	0.55	57	0.57	47	0.47	29	0.29
13	79	0.79	84	0.84	52	0.52	52	0.52	44	0.44	26	0.26
14	77	0.77	84	0.84	47	0.47	43	0.43	39	0.39	25	0.25
15	70	0.70	78	0.78	42	0.42	36	0.36	39	0.39	20	0.20
16	62	0.62	76	0.76	40	0.40	29	0.29	36	0.36	14	0.14
17	60	0.60	76	0.76	34	0.34	22	0.22	27	0.27	9	0.09
18	54	0.54	71	0.71	29	0.29	16	0.16	22	0.22	1	0.01
19	51	0.51	68	0.68	26	0.26	9	0.09	21	0.21	0	0.00
20	47	0.47	68	0.68	21	0.21	5	0.05	17	0.17		
21	44	0.44	63	0.63	17	0.17	2	0.02	16	0.16		
22	39	0.39	56	0.56	15	0.15	0	0.00	11	0.11		
23	36	0.36	51	0.51	12	0.12						
24	29	0.29	49	0.49	8	0.08						
25	24	0.24	47	0.47	4	0.04						
26	18	0.18	43	0.43	1	0.01						
27	12	0.12	39	0.39	0	0.00						
28	8	0.08	33	0.33								
29	2	0.02	26	0.26								
30	0	0.00	21	0.21								
			18	0.18								
			13	0.13								
			9	0.09								
			4	0.04								
			0	0.00								

Nx 1 = Survivors on day "x".

Lx = Survivors on day "x"/initial cohort.

The results from the studies on reproductive isolation, host preferences and adult survivorships supplement those from hybridization studies (KHASIMUDDIN & DEBACH, 1976). The significance of such biological distinctness among morphologically indistinguishable species populations has been expressed by DEBACH (1969) as follows: "From the standpoint of practical biological control, we are vitally interested in whether natural enemies differ from one another biologically, regardless of our ability to tell them apart morphologically. All grades of specific or subspecific genetically based differences may be important."

DOBZHANSKI & SPASSKY (1959) studied sibling species of *Drosophila paulistorum* DOBZHANSKY & PAVAN in Central and South America and found that bridging populations were present that produced fertile hybrids with other populations that were reproductively isolated from each other. They considered all these populations as representing a single species since gene exchange was possible among them through connecting links of bridging populations. The present situation appears entirely different, even though our information on possible bridging populations is largely lacking. However, the degree of isolation found is virtually complete in all cases. Therefore, gene exchange between these siblings is extremely unlikely to occur in nature should they occur sympatrically.

HALL *et al.* (1962), based on differences in host specificity, cross-mating and progeny production between a walnut aphid parasite, *Tryoxis pallidus* HALL, and a spotted alfalfa aphid parasite, *T. utilis* MUES., were able to demonstrate that both these morphologically similar species, which had been recently synonymized, were actually distinct sibling species.

Apparently, the 3 populations of *A. maculicornis* studied are actually biologically distinct sibling species and should be treated as such, especially for biological control purposes. Close observations on morphological features may in the future reveal differences that may help systematists to describe and rename each of the 3 siblings.

RÉSUMÉ

État biosystématique de trois populations allopatriques
(de *Aphytis maculicornis* [*Hym. : Aphelinidae*])

Cette étude concerne trois groupes de populations allopatriques de *Aphytis maculicornis* indistinguables du point de vue morphologique — « Perse », « Pakistan » et « Californie ». Des tests d'accouplement à choix multiples ont essentiellement indiqué un isolement complet de ces groupes pour la reproduction. Des expériences qui comprenaient six différentes espèces de cochenilles ont démontré qu'il existait des différences significatives de préférence d'hôte parmi les trois populations. Des tests déterminant la longévité des adultes ont également indiqué des différences significatives d'une population à l'autre, surtout à une température de 27°C. On en conclut que ces populations représentent trois espèces sœurs bien distinctes. Ces résultats soulignent l'importance de telles espèces sœurs dans le domaine de la lutte biologique.

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