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ORIGINAL PAPER

Male behaviors reveal multiple pherotypes within vine mealybug *Planococcus ficus* (Signoret) (Hemiptera; Pseudococcidae) populations

Hofit Kol-Maimon · Anat Levi-Zada · José Carlos Franco · Ezra Dunkelblum · Alex Protasov · Miriam Eliyaho · Zvi Mendel

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Abstract The vine mealybug (VM) females collected in Israel produce two sex pheromone compounds: lavandulyl senecioate (LS) and (*S*)-lavandulyl isovalerate (LI). The males display ambiguous behavior to LI: repulsion in the vineyard and attraction of laboratory-reared males. We addressed the question of individual male behavior, i.e., do males respond to both LS and LI, or might they display a distinct response to each of the two pheromone compounds. We compared male pherotype frequencies between wild-caught and laboratory-reared populations. Then, we examined the relationship between pherotype composition and male capture rates in pheromone traps. Finally, we addressed the heredity of the pherotypes. The Israeli VM populations contain nine different male pherotypes, as defined according to the male behavior to pheromone compounds. The studied Portuguese populations included five of the nine pherotypes; none of the Portuguese males were attracted to LI. It seems that the high frequency of males that were attracted to LI is related to dense VM populations. It is hypothesized that selection for the male pherotypes, I males, those that respond to LI, occur under high-density rearing conditions. This may result from shorter development times of males and females that produce more I male pherotypes. The lower relative frequency of trapping of males in LI-baited traps than expected from the percentage determined in a Petri dish arena suggests that males that respond solely to LS (S males) are better fliers. The results also suggest that the pherotype trait is inherited by both sexes of the VM.

Keywords *Planococcus ficus* · Sex pheromone · Pherotype · Polymorphism · Behavior · Heredity

H. Kol-Maimon · A. Levi-Zada · E. Dunkelblum · A. Protasov · M. Eliyaho · Z. Mendel (✉)
Department of Entomology, Volcani Center, ARO,
Bet Dagan 50250, Israel
e-mail: zmendel@volcani.agri.gov.il

J. C. Franco
Departamento de Protecção de Plantas e de Fitoecologia/Centro de Estudos Florestais, Instituto Superior de Agronomia,
Universidade Técnica de Lisboa,
1349-017 Lisbon, Portugal

Introduction

Structural differences among sex pheromones of different individuals belonging to the same species have been recognized in *Drosophila* spp. (Diptera) (Ferveur et al. 1996) and in one species of scarab beetle (Coleoptera) (Robbins et al. 2008), and mainly in moths (Lepidoptera) (Collins and Cardé 1989; Guerin et al. 1984; Hansson et al. 1987; Hill et al. 1982). In moths, however, use of different blend ratios is not just species-specific but also differ between strains or races, an aspect that has been extensively studied in the case of the European corn borer, *Ostrinia nubilalis* (e.g., Klun and Maini 1979; Linn et al. 1997). The term pherotype was applied to insects by several authors, to distinguish between different geographical strains of Lepidopteran species (Bontemps et al. 2004; Eizaguirre et al. 2003; Frérot et al. 1997; Malausa et al. 2007; Steven et al. 2001). In this study, we use the term 'pherotype' to describe conspecific males of the vine mealybug (VM), *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) that differ in their responses to the female sex pheromone (Zada et al. 2008).

The sex pheromone of the VM was identified as a single-component (*S*)-lavandulyl senecioate (LS) in females

originating from Californian vineyards (Hinkens et al. 2001), whereas VM females collected in Israeli vineyards produced both LS and (*S*)-lavandulyl isovalerate (LI) (Zada et al. 2003). It was found that laboratory-reared males were similarly attracted to both compounds. On the other hand, in the vineyard wild-caught males responded only to LS, and male captures in pheromone traps were often reduced when LI was added to the bait (Zada et al. 2003). In a further study, we found that the level of inhibition varied between plots and years (Zada et al. 2008).

The present study was conducted to elucidate the ambiguous behavior of the males of the VM. Since this behavior was determined on the population level, the first objective focused on what was happening at the individual level, i.e., do males respond to both LS and LI, or might they display a distinct response to each of the two pheromone compounds? The second objective concerned the similarity between male pherotype frequencies in wild-caught and laboratory-reared populations. The third objective concerned the relationship between pherotype frequency in a given VM population, as determined by laboratory tests, and male capture rates in pheromone traps baited with LS and LI at the population site. The fourth objective addressed the heredity of the pherotypes.

Materials and methods

Tested populations

Gravid females and ovisacs of the VM were collected in eight vineyards and a small fig grove in Israel, and in two vineyards in Portugal (Table 1). The sons of these females were considered as feral males in laboratory experiments, and males of the progeny of each feral cohort were considered as the first laboratory generation. Several generations (up to six) of each population were reared in the laboratory. A generation was determined 'from egg to egg'.

Characterization of VM density in the sampled vineyards

Estimates of the population density of VM (from second instar nymphs to ovipositing females) were supplied by the local Extension Service officers. The mealybug density (number of mealybugs per plant) was estimated by direct observation of vine stems and arms, leaves and bunches. The sampling procedure usually consisted of six counts during a 5-min period for each of the plant parts. Observations were performed by two persons with each one doing three counts per site. A total of 25–30 vines were sampled at each site. Low density, medium density and high density were defined as <5, 5–10 and >10 mealybugs per plant, respectively. The Pearson chi-square test was used to compare pherotype frequency of the populations within each group (Israel low, Israel high, and Portugal). Multivariate ANOVA was applied to contrast each pherotype frequency among the three groups of population. Throughout the study significance was set as $P<0.05$.

Production of males for the tests

VM cohorts were reared on potato sprouts in rearing chambers in darkness at 25°C. Male prepupae and pupae were manually separated from mealybug colonies or from folded tissue paper strips placed at the bottom of plastic ventilated cages in which the VM-infested potato sprouts were placed. Most of the males tended to leave the sprout and pupate in the paper folders at the end of second instar stage. They emerged as adults after 5–7 days and reached maturity 1–2 days after emergence, when the abdomen terminal setae had reached their maximum length (Mendel et al. 2008; Silva et al. 2009).

Table 1 Details on the areas where vine mealybug populations were sampled in Israel and Portugal

Collecting site	Coordinates	Elevation	Crop and variety	Population density
Israel				
Odem	33°44′N, 35°15′E	1,022	GV-Chardonnay	Low
Yonatan	32°77′N, 35°46′E	557	GV-Chardonnay	Low
Avne Eitan	32°26′N, 35°44′E	387	GV-Chardonnay	High
Qeshet	32°23′N, 35°88′E	723	Fig trees, *Ficus carica*	High
Bloom	32°07′N, 35°33′E	370	GV-Superior	High
Baco	33°28′N, 35°20′E	420	GV-Chardonnay	Low
Lachish-1	33°31′N, 34°44′E	242	GV-Superior	High
Lachish-2	33°31′N, 34°44′E	242	GV-Superior	Low
Portugal				
Tavira	37°6′N, 7°39′W	315	GV-Collection	Low
Pinhão	41°10′N, 7°33′W	327	GV-Chardonnay	Low

Synthesis of pheromones

We synthesized the pheromone components of VM and of the citrus mealybug (CM) *Planococcus citri* (Risso), (1*R*)-*cis*-2,2-dimethyl-3-isopropenyl-cyclobutanemethanol acetate (Bierl-Leonhardt et al. 1981) according to Zada et al. (2003, 2004), respectively. The racemic pheromone compounds were used for trapping males in the study plots, whereas the chiral stereoisomers were used in the laboratory tests. The chiral LS and LI were prepared from commercial racemic lavandulol (Zada et al. 2003). The (*S*)-LS and (*S*)-LI enantiomers were prepared from *R*- and *S*-lavandulol. The enantiomeric purity of (*S*)-LS and (*S*)-LI was 95–96% (Zada and Harel 2004; Zada and Dunkelblum 2006). The purity of the chiral esters was based on the enantiomeric purity of the chiral isomers of lavandulol that were obtained by enzymatic resolution of racemic lavandulol and subsequent esterification (Zada et al. 2003).

Laboratory arena

Male pherotypes were characterized according to their specific responses to LS, LI and CM pheromone. CM was used as a control to differentiate between VM males and possible CM males that might contaminate the cohort. Individual males were exposed to these compounds in no-choice tests, in which the compounds were presented in a random succession of three arenas. The pheromone solutions for the bioassay were prepared by dissolving the appropriate pheromone component, LS, LI, or CM, in *n*-hexane to a final concentration of 10 ng/μl. Males were bioassayed in 10-cm-diameter glass Petri dish arenas, at a pheromone dosage of 10 ng impregnated in a filter paper disk (5-mm-diameter, double-layer Whatman No 1), with two untreated paper disks as controls in each arena.

Pherotype characterization

Each test included 40–50 males; each individual was kept in each arena for a maximum of 10 min; usually, the response to each of the tested pheromones was recorded after 30–60 s. The tested males were allowed to rest for few minutes between successive exposures. The sequence of exposures was varied randomly. Three modes of responding behavior were defined: attraction, repulsion and indifference. Attraction (+) was characterized by fast movements of the male in a more or less direct line towards the pheromone bait, usually within less than 1 min. Repulsion (−) was characterized by an initial tendency to move towards the bait, followed by a rapid movement away from the impregnated disk, and then displaying typical restless movements, that in many cases included attempts to fly away from the pheromone source. Indifference (•) was noted when the male ignored the pheromone source, displaying random movement in the area, with no apparent difference between interception with the pheromone-impregnated disk and with one of the control disks. Excluding the rare cases of males that were attracted to the pheromone of CM, all other males were characterized according to the type of response to both LS and LI. Thus, we could expect nine male pherotypes based on behavioral responses (Table 2).

In the analysis of the results we clustered the pherotypes into four groups: I—those attracted to LI alone, i.e., I(+)S(•) and I(+)S(−); S—those attracted to LS alone i.e., I(•)S(+) and I(−)S(+); IS—those attracted to both LS and LI, i.e., I(+)S(+); and N—those that displayed no attraction to either pheromone compounds, i.e., I(•)S(•), I(•)S(−), I(−)S(•) and I(−)S(−). The N males were ready to mate as they made physical contact with a female. Pearson's chi-square analysis ($P<0.05$) was applied to compare pherotype frequency of the reared populations within each group (Israel low, Israel high and Portugal). Multi-way ANOVA ($P<0.05$) was applied to contrast each pherotype frequency among the three groups of population.

The analyses in all tests in the study were conducted by means of the JMP software, version 8.0.2 (SAS Institute 2008).

Changes in pherotype frequency as a function of the number of generations in laboratory-reared populations

Pherotype frequency was determined in two low-density populations (Odem and Yonatan) and two high-density populations (Avne Etan and Lachish-1) in Israel. We

Table 2 Nine possible combinations of vine mealybug male pherotypes, characterized according to their responses to (*S*)-lavandulyl senecioate and (*S*)-lavandulyl isovalerate

Response toward (*S*)-lavandulyl isovalerate = I	Response towards (*S*)-lavanduyl senecioate = S		
	Attraction (+)	Repulsion (−)	Indifference (•)
Attraction (+)	S(+)I(+)	S(−)I(+)	S(•)I(+)
Repulsion (−)	S(+)I(−)	S(−)I(−)	S(•)I(−)
Indifference (•)	S(+)I(•)	S(−)I(•)	S(•)I(•)

studied the pherotype frequency among males collected in the field (as ovisacs or gravid females) (first generation), and the male pherotypes of the laboratory-reared third and sixth generations. Rearing of the two low-density populations in the sixth was not achieved due to a technical problem). Pherotype frequency between generations for each population density was compared using the *G* test.

Comparison of pherotype occurrence frequencies and male capture rates in traps baited with LS and LI pheromones for a given vineyard

The ratios between VM pherotype groups in four populations in Israel (Bloom, Baco, Lachish-1 and Qeshet) were determined in laboratory tests for sons of gravid females, or egg masses that were collected in each location (Table 1). The ratio between the pherotype groups in each population was determined in laboratory tests for sons of gravid females (or as egg masses) that were collected in each location. In this case, we assumed that males of the set LS consisted of males defined as S group and half of those males defined as IS group. The other set—LI—consisted of males defined as I group and half of those defined as IS group. The N pherotype group was not considered. In this case, based on their behavior in the laboratory, we acted upon the assumption that males of the IS group in the field were equally captured in traps baited with LS and LI, whereas the males of the N group were not trapped. In each location, after collection of the gravid females or egg masses, we set up delta traps baited with LS or LI, or without bait. A sticky plate, 16×9.5 cm, was placed at the bottom of each trap. The traps were baited with 50 μg of the pheromone compound, impregnated in a rubber disperser (Yogev, Rishon LeZion, Israel). Two replicates were used per treatment in each density, and the traps were exposed for 2 weeks during the summer. For each replicate, we compared the ratio between the number of males of 'LS' and 'LI' sets obtained in the Petri dish arena in the laboratory with that between the numbers of males captured in traps baited with LS- and LI-baited traps in the field. The frequency occurrence of the pherotypes as obtained by pheromone traps and determined in the laboratory arena was compared using the *G* test for homogeneity with replicates.

Effect of father pherotype on the son's pherotype

Groups of 30 virgin females of the fifth laboratory generation (gravid females of the original population were collected at Odem vineyard), randomly removed from the rearing at the third instar nymphal stage, and divided into three subgroups of ten females. Later, at the stage of young adult, each group of females was exposed to 12 to 16 males of S, I or N pherotypes, and the corresponding progeny was used to determine son pherotype frequency (S, I, IS or N) for each subgroup. Since all male pherotypes occurred in the tested population, we assume that the females were heterozygous, but that was not expected to constrain the results. The effect of the fathers' pherotype on the distribution of the sons' pherotype was analyzed using Pearson's chi- square test.

Development patterns of various male pherotypes

Emergence patterns of adult VM males, sons of ten randomly selected singly reared females of the sixth laboratory-reared generation, arranged according to the four pherotype groups (I, S, IS, and N) were recorded. The male progeny of each female were removed every other day during 20 days, i.e., a total of a ten collection intervals. Male of each collection interval (first, second, …, tenth) was kept in a separate Petri dish. Mature adult males, i.e., those whose abdominal terminal setae had attained maximum length, were characterized according to their pherotype trait and time of their emergence interval (interval 1 consisted of emerging adult males that were collected during the first 2 days of emergence, interval 2 consisted of emerging adult males that were collected during the during the third day and fourth day of emergence, and so forth). The effect of the emergence interval on the distribution of male pherotypes was analyzed by the Pearson chi-square test.

Effect of female development time on their sons' pherotypes with respect to the grandfather pherotype

The son pherotype, divided into I, S, IS and N groups, was determined according to their mother's development times and the pherotypic characters of their grandfathers. These mothers were daughters of 15 females that had been randomly collected at the third instar nymphal stage from laboratory-reared VM. The 15 females were divided into three groups of five, and each group was exposed to 6–8S, I or N pherotype males. The first five early-developing daughters, i.e., the first five to start the oviposition process, and the last five late-developing daughters, i.e., the last ones to start the oviposition process, were selected from each of the above-mentioned groups (i.e., two daughter subgroups of each group of five mothers). Two subgroups of daughters, 'early-developing' and 'late-developing' daughters, of each of the three mother groups (a total of six subgroups), were separately allowed to mate with randomly collected males. The distribution of the four pherotypes among the grandsons was determined separately for each of the two subgroups. The effect of 'grandfather pherotype' and 'mother development time' on the distribu-

tion of grandsons pherotype was analyzed between treatments using the *G* test.

Results

Male pherotype characterization

The observations revealed males of all nine expected pherotypes (Table 2) that display the nine possible combinations of responses to LS and LI. The frequency distribution of the nine male VM pherotypes identified among 2,796 tested males whose mothers were collected in Israeli vineyards or from various generations reared on potato sprouts in the laboratory is displayed in Table 3. All nine male pherotypes were present in various percentages among sons of both laboratory-reared females and field-collected females. The percentage of the I pherotype was significantly higher among male offspring of laboratory-mated females than among sons of mated females collected in the vineyard ($\chi^2_{(df=1)}=22.298$, $P<0.0001$). Consequently, as expected, the percentage of S pherotype males was significantly higher among offspring of field-mated females than among those of laboratory-mated females ($\chi^2_{(df=1)}=10.117$, $P=0.0015$). The percentage of the N pherotype was not significantly different between laboratory and field males ($\chi^2_{(df=1)}=0.026$, $P=0.8713$). Overall, the pherotype I(−)S(+) occurred in the highest percentage: 28.7%. The frequency of occurrence of male pherotypes among the 135 laboratory-reared males from Tavira, Portugal, showed only five pherotypes among this population. All belonged to the S and N groups, in the following descending percentages: I(−)S(+), 63.7%; I(•)S(+), 25.7%; I(−)S(•), 6.2%; I(−)S(−), 3.5%; and I(•)S(•), 0.9%.

Frequency of occurrence of pherotype groups in studied field locations

The male pherotype compositions as affected by density (vineyards in eight locations in Israel with low-density or high-density VM populations) and origin (two VM populations locations in Portugal) are compared in Fig. 1. Whereas the vineyards with low-density VM populations (Odem, Yonatan, Lachish-2, and Baco) displayed low percentages of pherotypes that are attracted to LI (5–12%), i.e., I and IS, and high percentages of the S pherotypes (54–76%), the other three Israeli vineyards and the Israeli fig grove (in Qeshet) displayed high percentages of I and IS pherotypes (45–70%), and a relatively wide range of percentages of the S pherotypes (19–62%). Both I and IS pherotypes were absent from the two Portuguese populations of VM. Males of the N pherotype were present in various ratios in all the studied populations. The pherotype frequency distributions varied significantly between populations within the Israel high and the low density groups ($\chi^2_{(df=3)}=216.6$, $P<0.0001$; and $\chi^2_{(df=3)}=38.5$, $P<0.0001$, respectively), but not between the two Portuguese populations ($\chi^2_{(df=1)}=0.25$, $P=0.61$). Among the two Israeli population groups, the percentage of males that were attracted to LI was significantly higher ($\chi^2_{(df=1)}=28.82$, $P=0.0001$) in the high density populations (>10 mealybug/plant) than in the low density populations (<5 mealybug/plant) of VM. The percentage of males that were attracted to LS was significantly lower ($\chi^2_{(df=1)}=18.61$, $P=0.0007$) in the high density populations than in the low density populations. The results mean that male pherotypes attracted to LI were absent in the tested Portuguese populations. Conversely, in Israel these pherotypes were common among VM high populations and infrequent at low VM population density.

Changes in pherotype composition between laboratory generations

Results, which are displayed in Fig. 2, reveal a significant effect of the number of generations the population was reared in laboratory on the pherotype frequency distributions ($\chi^2_{(df=4)}=45.41$, $P<0.0001$). In contrast, no significant effect of the origin on the pherotype frequency distributions was found ($\chi^2_{(df=2)}=5.2$, $P=0.07$). In the two low density

Table 3 Frequency distribution (%) of the nine types of vine mealybug male pherotypes (see Table 2) identified among tested males, whose mothers were collected in Israeli or Portuguese vineyards or developed through various generations on potato sprouts in the laboratory

Pherotype group	Pherotypes	Israel (Total of 2796)	Portugal (total of 113)
I	S(•)I(+)	4.65	0
I	S(−)I(+)	6.87	0
S	S(+)I(•)	18.96	25.66
S	S(+)I(−)	28.72	63.72
IS	S(+)I(+)	16.02	0
N	S(•)I(•)	9.05	0.885
N	S(−)I(−)	10.55	3.54
N	S(•)I(−)	4.61	6.195
N	S(−)I(•)	0.57	0

The pherotypes are arranged according to the four groups

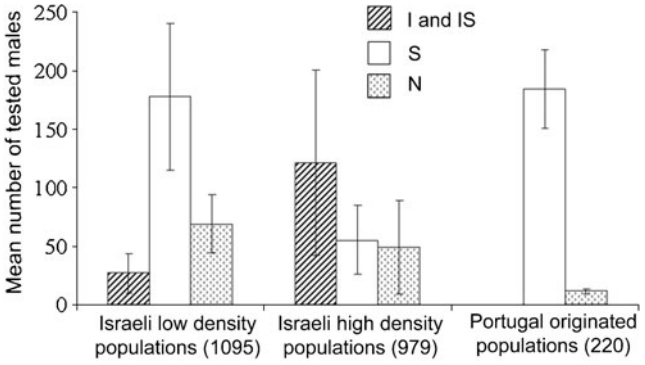

Fig. 1 Pherotype frequency distribution as affected by density and origin. *Numbers in brackets* are the total number of tested males for each category. Low-density populations are defined as a population with less than five adult females/vine. Males of each population were arranged in three groups of pherotypes: I + IS—males attracted to LI or both pheromones LI and LS, S—males attracted to LS alone, N—males not attracted to either of the pheromones. No LI attracted males was observed in Portuguese populations

populations (Odem and Yonatan), the high percentages of S pherotypes and low percentages of I and IS pherotypes did not significantly change between the first and third generations (82% and 76% for the S pherotypes, $\chi^2_{(df=1)}=0.88$, $P=0.35$; 11% and 5% for the IS + I pherotypes ($\chi^2_{(df=1)}=3.13$, $P=0.08$). A significant increase in the N pherotype was obtained between the first and the third generations from 5% to 21% ($\chi^2_{(df=1)}=7.23$, $P=0.007$). In the two high density populations (Avne- Eitan and Lachish-1), the frequency of S pherotypes steeply decreased from the 36% in the first generation to 8% in the sixth generation ($\chi^2_{(df=1)}=5.7$, $P=0.02$, from the first to the third generations; and $\chi^2_{(df=1)}=8.08$, $P=0.005$, from the third to the sixth generation). The frequency of IS + I pherotypes moderately fluctuated between the tested generations (which ranged between 45% and 61%) with significant differences between the first and the third generations, but not between the third and the sixth generations ($\chi^2_{(df=1)}=7.9$, $P=0.005$, from the first to the third generation; and $\chi^2_{(df=1)}=4.9$, $P=0.06$, from the third to the sixth generation). A significant increase in the N pherotype was obtained between the first generation and the third and sixth generations, increasing from 13% to 35% (from the first to the third generation, $\chi^2_{(df=1)}=0.8$, $P=0.38$; and from the third to the sixth generation, $\chi^2_{(df=1)}=25.05$, $P<0.0001$). The results mean that the major shift in male pherotype composition during several laboratory rearing was an increase in the percentage N pherotypes and a decrease in the percentage of S pherotypes.

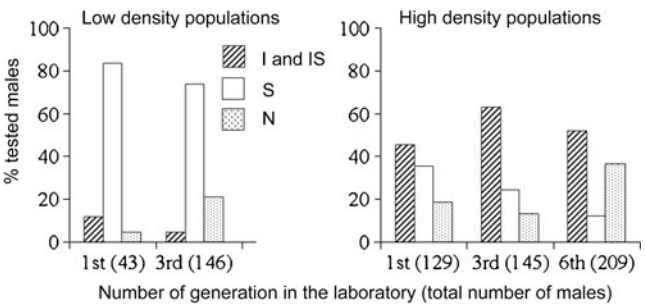

Fig. 2 Frequency distribution of male pherotype groups of Israeli populations as related to the population density level (low, Odem, Yonathan, and high, Avne Eitan and Lachish-1) and the number of generations in the laboratory (*numbers in parentheses*: total number of tested males). Males of each population were arranged in three groups of pherotypes: I and IS group—males that respond only to (*S*)-lavandulyl isovalerate (LI) and to both I and (*S*)-lavandulyl senecioate (LS); S group—males that responded only to LS; and N group—males that did not respond to either LI or LS

Comparison of pherotype composition and male capture in traps baited with LS and LI for a given vineyard

The results (Fig. 3) reveal a significant effect of population density on pherotype frequency distributions ($\chi^2_{(df=1)}=205.3$, $P<0.0001$). In addition, a significant effect of pherotype diagnosis (traps vs. arena) on pherotype frequency distributions was found ($\chi^2_{(df=1)}=109.5$, $P<0.0001$). The percentage of I males among high density populations was significantly lower than the percentage of S males among trapped males as compared with arena diagnosed ones ($\chi^2_{(df=1)}=403.2$, $P<0.0001$). This is supported by the fact that the percentage of I males among low density populations was also significantly lower than the percentage of S males among trapped males compared with arena

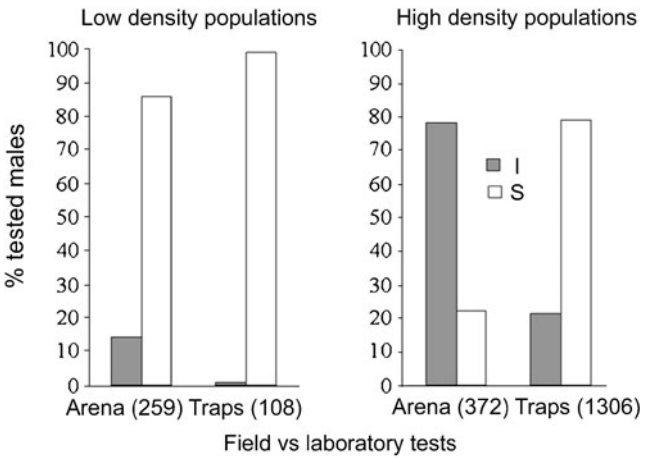

Fig. 3 Frequency distribution of VM males sampled in two low-density populations (Lachish-2 and Bacu) and two high-density populations (Bloom and Qeshet). The pherotype frequency were determined according their attraction to (*S*)-lavandulyl isovalerate (I) and (*S*)-lavandulyl senecioate (S) tested in laboratory arena and by sticky traps baited with these compounds that were set up in the respective locations of origin for 2 weeks (see "Materials and methods" section). The males tested in the laboratory were the offspring of mated or ovipositing females collected in the respective locations. (*Numbers in parentheses*: total number of tested or trapped males)

diagnosed ones ($\chi^2_{(df=1)}$=20.5, P<0.0001). The results mean that male pherotypes attracted to LS were captured by pheromone traps in higher proportions than their actual percentages in the tested VM population, as revealed by their behavior in the laboratory areas. Males attracted to LI displayed an opposite trend; they were captured in lower proportions than their actual percentages in the tested populations.

Heredity of male pherotypes

The pherotype frequency of sons of females that were mated with S or I males differed from that in the source population (Fig. 4). Mating with N males resulted in a pherotype frequency distribution similar to that of the source population, whereas mating with S males increased the percentage of S pherotype in offspring from 11.0% to 13.2%, and N pherotype from 22.4% to 33.3%; decreased those of I from 33.2% to 18.8%; meanwhile, the percentage of IS pherotypes remained unchanged. Mating with I males reduced the percentage of S pherotype offspring from 11.0% to 4.0% and increased those of I from 32.5% to 39.4%; the percentage of IS and N pherotypes remained unchanged. Mating with N males reduced the percentage of I pherotype offspring from 32.5% to 18.6% and increased those of IS from 34.1% to 47.5%; the percentage of S and N pherotypes remained unchanged. No significant differences were found between the percentages of N pherotypes, son of S, I or N fathers and source population ($\chi^2_{(df=3)}$= 1.38, P=0.71).

The percentages of I male pherotypes, sons of S and N fathers, were significantly lower than that of the pherotype I male group in the source population ($\chi^2_{(df=3)}$=54.43, P< 0.0001 and $\chi^2_{(df=3)}$=15.81, P=0.001, respectively). The percentages of I and N male pherotypes that were sons of I fathers did not differ significantly from the I and N sons of the source population ($\chi^2_{(df=1)}$=3.18, P=0.08 and $\chi^2_{(df=1)}$= 0.07, P=0.8, respectively). The results mean that mating with I and S father pherotypes increases the frequency distribution of I and S pherotypes among their sons, respectively. Moreover, mating with N father pherotypes increases the frequency distribution of their IS sons' pherotypes.

Development pattern of various male pherotypes

The emergence pattern of adult male VM, with respect to each 2-day emergence interval of the tested population, is displayed in Fig. 5. A total of 952 males were collected during a 3-week period for the ten emergence intervals; all were tested for their pherotype behavior. The two early intervals (1–2) and the three latest intervals (8–10) consisted of 19–59 males; the middle intervals (3–7) consisted of 107–251 males (Fig. 5). There were no S pherotype males in the two early intervals, and no I and N pherotype males in the last (tenth) interval. The percentage of S males was significantly higher among the two last intervals than in the two early ones (19% and 36% compared with 0% and 0%; $\chi^2_{(df=1)}$=19.3, P<0.0001). The percentage of I males in the first interval compared with the last interval was not significantly different (6% compared with 0%; $\chi^2_{(df=1)}$=1.6, P=0.21), probably due to their low numbers in these particular collections. The percentage of N males in the first interval compared with the last interval was significantly different (17% compared with 0%; $\chi^2_{(df=1)}$=5, P=0.03). IS pherotype groups were present in all emergence intervals. The percentage of IS males was significantly lower in the fifth and the sixth intervals compared with the other intervals (35% and 37% compared with 53–78%; $\chi^2_{(df=1)}$=52.6, P<0.0001). The results mean that there was a change in the frequency of I

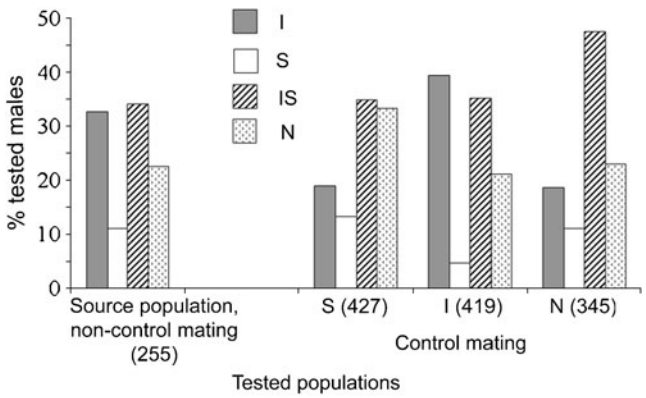

Fig. 4 Effects of I, S and N father pherotypes which were mated with females of the first two laboratory generations (see "Materials and methods" section) on the frequency distribution of pherotype groups of their sons, as compared with the original population (collected in Odem vineyard). *Numbers in parentheses* correspond to the number of tested males

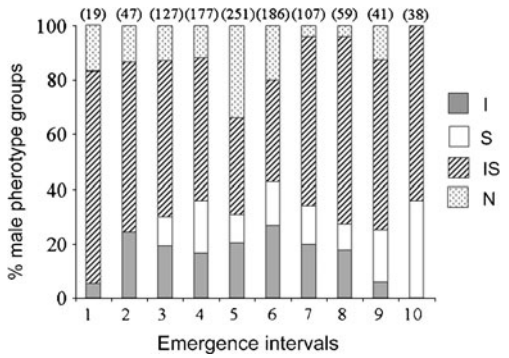

Fig. 5 Emergence patterns of vine mealybug males, sons of randomly selected females of the sixth generation of laboratory rearing, arranged according to four groups of pherotypes: I, S, IS and N as related to their emergence interval of 2 days (see "Materials and methods" section). *Numbers in parentheses* are the numbers of tested males for each emergence intervals

and S pherotypes during the emergence period. The frequency of I was steady until near the end of the emergence period and they were absent at the end. The S pherotypes were absent at the beginning of emergence; their occurrence remained steady until near the end of emergence when their proportion significantly increased.

Effect of VM female development time on their sons' pherotype

An apparent effect of the development time of females on the pherotype composition of their sons is displayed in Fig. 6. Regardless of the effect of the father pherotype, the results revealed a significant effect of the development time of females (daughters) on the pherotype composition of their male offspring (grandsons) ($\chi^2_{(df=3)}=27.28$, $P<0.0001$). In addition, regardless of the effect of the development time of females (daughters), a significant effect of the grandfathers' pherotype on the pherotype composition of their grandsons was also determined ($\chi^2_{(df=6)}=22.26$, $P=0.001$). Regardless of the grandfather's pherotype, the percentages of I males were 38.7±14.1% (mean±SD) and 22.0±9.3% among the sons of early- and late-developing females, respectively. The percentages of S males displayed an opposite trend: 13.2±3.4% and 33.80±9.8% for early- and late-developing females, respectively. The percentage of I male offspring was significantly higher than that of S males among the sons of early-developing females ($\chi^2_{(df=1)}=14.42$, $P=0.0001$) and significantly lower than that of S males among the sons of the late-developing females ($\chi^2_{(df=1)}=24.7$, $P<0.0001$). The differences among the IS and N pherotype groups were much smaller and not significant in the studied population: 33.2±12.1% vs. 28.8±8.2% and 14.9±1.7% vs. 15.4±4.5% for IS and N pherotypes, respectively. No significant differences were found between late- and early-developed females in the percentages of IS and N male offspring ($\chi^2_{(df=1)}=0.68$, $P=0.41$ and $\chi^2_{(df=1)}=0.0$, $P=0.99$, respectively).

The results mean that there was a clear effect of the development time of females, with or without the effect of the grandfathers' pherotype on the pherotype composition of their sons, particularly in the ratio of the I and S son pherotypes. The percentage of I pherotype sons was much higher than that of S pherotype sons of early-developing females and vice versa for late-developing females.

Regardless of the development timing of the females (daughters), the percentages of I males were 39.2±21.5%, and 19.5±10.8%, and 32.3±3.1% for I, S and N grandfathers, respectively; and the percentage of S males displayed a different pattern, i.e., 24.5±17.2%, 29.4±17.7% and 16.6±8.9% for I, S and N grandfathers, respectively. The percentages of IS males were 19.5±0.2%, and 37.2±2.5%, and 36.4±7.1% for I, S and N grandfathers, respectively; the percentages of N males displayed a different pattern, i.e., 16.9±4.0%, 13.9±4.4% and 14.7±1.3% for I, S and N grandfathers, respectively. The percentage of I male offspring was significantly higher than other male offspring pherotypes among grandfathers I and N compared with grandfathers S ($\chi^2_{(df=2)}=15.06$, $P=0.0005$). The percentage of S male offspring was significantly higher than other male offspring pherotypes among grandfathers S compared with grandfather I and N ($\chi^2_{(df=2)}=8.3$, $P=0.02$). The percentage of SI male offspring was significantly higher than other male offspring pherotypes among grandfathers S and N compared with grandfather I ($\chi^2_{(df=2)}=12.21$, $P=0.002$). The percentage of N male offspring was not significantly different than other male offspring pherotypes among grandfathers I, S and N ($\chi^2_{(df=2)}=0.88$, $P=0.64$). The results mean that there was a clear effect of I and S grandfather pherotypes, excluding the effect of development time of females, on the pherotype composition of their grandsons. I and S grandfathers produced significantly more I and S grandsons, respectively, than other three grandson pherotypes. This was not the case for the N grandfathers.

The percentages of I and S males were markedly different: 54% vs. 12% and 12% vs. 42% for earlier and

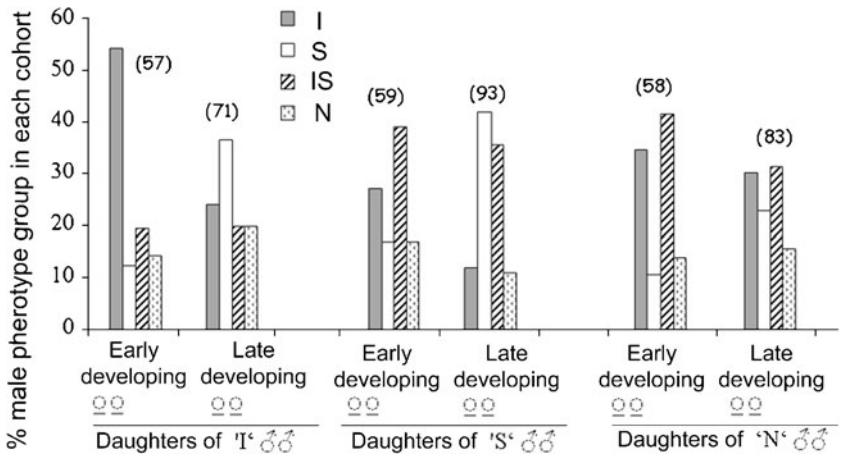

Fig. 6 Comparison of the effect of early-developing vs. late-developing females, daughters of a father of known pherotype (I, S or N) on their sons' pherotype group f requency distribution. The females were allowed to mate with randomly collected males of the same group

latter females, respectively. The percentage of I males was highest in early-developing female whose fathers were I, and lowest in late-developing females whose fathers were S than the other combinations ($\chi^2_{(df=5)}=33.33$, $P<0.0001$). The percentage of S males was highest in late-developing females whose fathers were S or I compared to the other combinations ($\chi^2_{(df=5)}=32.74$, $P<0.0001$). The percentage of IS males was highest in late-developing females whose fathers were S or I compared to the other combinations ($\chi^2_{(df=5)}=13.40$, $P=0.02$). The percentage of N males did not differ significantly between combinations ($\chi^2_{(df=5)}=2.87$, $P=0.72$). The results mean that the most distinct difference in male pherotype distribution was observed between the two 'extreme' combinations: sons of early-developing daughters of I males and sons of late-developing daughters of S males (Fig. 6).

Discussion

The Israeli VM populations contain nine different male pherotypes, as defined according to male behavior toward the sex pheromone compounds LS and LI. The results suggest that the attraction to LI is inherited in Israeli VM populations. The two Portuguese populations of VM include five of the nine pherotypes; the missing pherotypes would comprise males that are attracted to LI. The finding that some of the males displayed repulsion and/or indifference to one or both LS and LI compounds is surprising, and the ecological meaning of these behaviors is not clear. However, male inhibition by a geometrical isomer, (*Z*)-2-isopropyl-5-methyl-2,4-hexadienyl acetate, has been reported for the passion VM *Planococcus minor* (Maskell) sex pheromone (*E*)-2-isopropyl-5-methyl-2,4-hexadienyl acetate (Ho et al. 2007).

With respect to the emergence patterns of adult VM males, we found that the percentage of males attracted to LI is higher among the sons of early-developing females than among sons of late-developing females. The opposite occurred for the percentages of males that are attracted to LS, with relatively more males attracted to LS that were progeny of late-developing females. Based on these findings, we suggest that early-developing females will produce relatively more I males and less S males than their late-developing counterparts. The latter females display the opposite percentage of male pherotypes. This may indicate the existence of female pherotypes.

Our findings suggest that the high frequency of the occurrence of males that are attracted to LI is related to dense VM populations. Under laboratory rearing conditions, the population tends to alter its pherotype composition; our observations in the laboratory display a shift in the pherotype composition, mainly reduction of S and increase of N pherotypes. Population density is a major factor in natural selection, and individuals that adapt to high-density conditions become the dominant type (Sokolowski et al. 1997). We hypothesize that selection for I pherotypes should occur under laboratory rearing conditions that are characterized by high density, as well as under high density in natural situations. Adaptation to a changing environment should be associated with an increase of genetic diversity. Assortative mating between specific genotypes may further facilitate the differing modes of response to LS and LI by different male pherotypes, assuming that each pheromone compound of VM, LS and LI, may be produced by different female pherotypes. Hence, assortative mating may promote the change in the frequency of occurrence of genotypes in the population. The occurrence of a large percentage of VM males attracted to LI during periods of high population density may result from shorter development times of males and females that produce more I pherotypes. Furthermore, the higher percentage of I pherotypes in the male VM population compared with their relative frequency of occurrence in traps baited with LI suggests that S males are better fliers than I males. Following the same rationale, we suggest that the N pherotype males do not fly towards a pheromone source. Our results show that males of all four pherotype groups are fertile. Therefore, a high frequency of occurrence of I and N males in dense VM populations should be expected, because under high population density good flight capability may not be an advantage for males when females can be readily found even by walking. The energy costs and risks of flying is high (e.g., Rankin and Burchsted 1992; Shirai 1995; Walters and Dixon 1983) and, indeed, in the present study many of the I males did not reach the traps baited with LI. The putative superior flight, navigation or long-range sensing capability of S males would enable them to search for mates over long distances. The comparative advantage of this trait would be manifested particularly under low population levels, when females are scarce and widely spaced.

In light of these assumptions, we suggest that in laboratory culture, where mealybugs are reared in dense colonies, there is a shift in favor of high percentages of I, IS and N males, compared with that of S males. In populations from which the I and IS pherotypes are probably missing, such as the two studied Portuguese populations, and in California, where the LI compound was not detected among the local populations of VM (J. Millar, personal communication), we expect that the percentage of the N group would increase from generation to generation during laboratory rearing. Thus, whereas in the case of populations of the European corn borer, the occurrence of different pherotypes in France is related to host ramification under habitat pressure exerted by natural enemies (Thomas et al. 2003).

In the present case, the population density is the key factor in the alteration in pherotype composition.

The result of assortative mating in the present study suggests that the pherotype trait is inherited (see Figs. 4 and 6). The fact that early-developing females produced relatively more I males and less S males than late-developing females suggests that the pherotype trait also occurs among females. Not much is known about phenotypic trait heredity in scale insects, especially with regard to mealybugs. In most neococcoid scale insects, the mechanism of sex determination is haplodiploidy with paternal genome elimination (PGE), which means that males begin life as diploid zygotes but ultimately produce sperm that carry only their mother's genome (Normark 2003). In mealybugs (Pseudococcidae), the genome of paternal origin in the embryos that develop into males becomes heterochromatic and genetically inactive and is not transmitted to the offspring (Nur 1990). However, the rate of PGE in mealybug species and the inheritance role of mealybug males are unclear (Nur 1990). Our findings show that VM males influence the pherotype trait of their sons and grandsons, at least through their daughters. The father's ability to pass this trait to their grandsons through their sons is under study.

It seems that the development rate of male and female VM is linked to the pherotype trait: I males develop faster than S males. A correlation between the pherotype trait and development time trait was also shown in the European corn borer (Thomas et al. 2003). Such correlation may be the result of pleiotropy, when one gene regulates several traits, or it could be due to linkage disequilibrium when alleles from one site are influenced by alleles from other sites in the genome (Coyne and Orr 2004). We speculate that both the above-mentioned traits in the Israeli population of the VM are linked to production of specific pheromone components; that early-developing females produce LI, whereas late-developing ones mainly produce LS. Following the same rationale, we suggest that there might be several corresponding female pherotypes, i.e., females that produce only LS or LI (S and I groups, respectively), females that produce both LS and LI (IS group), and females that do not produce the sex pheromone (N group). We would expect the frequency of occurrence of both IS and N to increase in high-density populations.

Our findings suggest that the occurrence of phenotypic and genetic changes in the VM population during the transition from the latent to the epidemic phase might be ecologically parallel to what happens in locust populations (Chapuis et al. 2008) or moth populations (Schowalter et al. 1986; Simchuk et al. 1999). This may be true also for other mealybug species or even for other scale insects. One ecological advantage for a shift from production of LS to production of LI may be related to the presence of *Anagyrus* sp. near *pseudococci* (Hymenoptera; Encyrtidae), a common natural enemy of VM in the Mediterranean region (e.g., Triapitsyn et al. 2007). This parasitoid is attracted to LS, but despite the similarity between the structures of LS and of LI, no significant response of the parasitoid to the latter compound was observed (Franco et al. 2008). Therefore, in high population densities of the VM, when mortality caused by this parasitoid is expected to be higher due to a numerical response to host increase and the fact that the population is more exposed to natural enemies, the I pherotypes would have an advantage in relation to the IS and S ones.

The VM is considered of Palearctic origin (Miller et al. 2005), probably from the Mediterranean Basin (Ben-Dov 1994), although the precise area of origin is not clear. The diverse pherotype composition observed in Israeli VM populations in comparison to other allopatric populations (e.g., Portuguese) further supports the Middle East as its region of origin. Based on this hypothesis, the lack of the I and IS pherotypes in the case of the two Portuguese populations and that from California may be explained by the founder effect, displaying a relatively low genetic variation of the founders of a population during geographical expansion (Schowalter 2000).

Finally, the occurrence of male pherotypes that are attracted to LI should also be considered in schemes for monitoring and control of VM by means of pheromones.

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