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A Two-Component Female-Produced Pheromone of the Spider *Pholcus beijingensis*

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Abstract Chemical signaling plays an important role in spider sexual communication, yet the chemistry of spider sex pheromones remains poorly understood. Unlike insects and mammals, the identification of spider pheromones has seldom been attempted, and no multicomponent pheromones have been found. Empty webs of sexually receptive females of *Pholcus beijingensis* were more attractive to male conspecifics as compared to webs of sexually unreceptive females or to mature males. Coincidently, chemical analysis revealed that (*E,E*)-farnesyl acetate, diisobutyl phthalate, and hexadecyl acetate of the spider webs exhibited higher relative abundances in sexually receptive females than in sexually unreceptive females or males, indicative of possible pheromone components. Two-choice behavioral assays verified that the blend of (*E,E*)-farnesyl acetate and hexadecyl acetate (w/w: 2:1) attracted males at a dosage equivalent to the amounts of these compounds in one spider web, whereas neither compound alone aroused males. In addition, diisobutyl phthalate (a likely contaminant from contact with plastic) alone or in combination with either of the acetates did not evoke the males' attraction. The behavioral data suggest that (*E,E*)-farnesyl acetate and hexadecyl acetate comprise a two-component female-produced sex pheromone in *P. beijingensis*, the first multicomponent pheromone found in spiders.

Keywords (*E,E*)-Farnesyl acetate · Hexadecyl acetate · Sex pheromone · Spider web · Araneae · Pholcidae

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Introduction

Spiders generally are aggressive and cannibalistic. They have evolved elaborate means to communicate with each other during courtship. The exchange of chemical signals is probably the first type of communication in spiders that bring the males and females together (Weygoldt 1977). Chemical signaling plays an important role in spider sexual communication, and contact between the emitter and the receiver is not necessary (Gaskett 2007). Silk-bound pheromones are important in courtship. For example, in some species, a mate-searching male rapidly cuts the thread of a virgin female's web or packs the silk into a tight mass. This behavior may hinder evaporation of the sex pheromone thereby reducing the chances of another male reaching the web (Watson 1986; Schulz and Toft 1993). Male wolf spiders, *Schizocosa ocreata,* show significantly more and longer bouts of chemosensory and courtship behaviors on the silk of adult, unmated female spiders than on any other stimulus treatment (Roberts and Uetz 2005).

Silk-bound sex pheromones occur in several arachnid families (Dondale and Hegdekar 1973; Suter and Renkes 1982; Searcy et al. 1999; Michael and Maydianne 2004; Roberts and Uetz 2004, 2005). However, the chemistry of these remains poorly understood, especially in comparison with those of crustaceans and insects, and some mammals, reptiles, and fishes. There are only three species of spiders for which pheromones have been identified. The pheromone compounds (*R*)-3-hydroxybutyric acid (HBA) and its dimer, (*R*)-3-[(*R*)-3- hydroxybutyryloxy]-butyric acid (HBBA), occur in a sheet-web spider *Linyphia triangularis* (Schulz and Toft 1993). The wandering spider, *Cupiennius salei*, and the orb-web spider, *Agelenopsis aperta*, have two other sex pheromones: (*S*)-1,1′-dimethyl citrate (Papke et al. 2000; Tichy et al. 2001) and 8-methyl-2-nonanone (Papke et al. 2001). Structurally unrelated to other known pheromones, both HBA and (*S*)-1,1′-dimethyl citrate seem to be unique to spiders. The pheromone 8-methyl-2-nonanone resembles a component of insects, that include the caddisfly, *Hesperophylax occidentalis,* and the Asian palm weevil, *Rhynchophorus ferrugineus* (Hallett et al. 1993; Bjostad et al. 1996). Herein, we explored the structure, function, and chemical mechanism(s) of sexual communication in spiders.

Pholcid spiders (Araneae, Pholcidae) often are found in houses and buildings, especially in basements and cellars. *Pholcus beijingensis* is common in various caves in the vicinity of Beijing. They usually spin untidy webs in corners or on the stone walls of cave entrances. Sexual selection occurs in pholcids (Uhl 1998; Uhl et al. 2005). The species is polygamous, and males and females alike have multiple mating partners. During the reproductive season, males abandon their webs to search for potential mates, while the females wait on their webs for males. The approach of a mature male rarely triggers predatory or aggressive behavior in sexually receptive females. In natural populations, immature and adult spiders overlap during much of the active breeding season (Chen and Li 2005; Chen et al. 2008). Selection may favor males that distinguish conspecific, sexually receptive females during the reproductive season, otherwise they would be eaten. We isolated chemical signals from the web thread of *P. beijingensis*, and identified the sex pheromone components.

Methods and Materials

Subjects

Juvenile and adult *P. beijingensis* were collected in March and May of 2007 at the entrance of a bat cave located southwest of Beijing, China (39º42.350′N, 115º42.825′E). Each spider was kept in a glass cuvette (4 cm i.d.×12 cm high) with a small moistened wad of cotton on the bottom to provide humidity. Cuvettes were put in a climatic chamber (RXZ-268B, Ningbo Jiangnan Instrument Factory) under a 14:10 h (L/D) photoperiod regime at 25°C (day) and 22°C (night). About 10–15 fruit flies (*Drosophila melanogaster*) were provided to each spider for food once a week.

Test for Attraction to Silk of Females

We screened the following classes of *P. beijingensis*: juveniles, sexually receptive females, egg-holding females, and mate-searching males. To determine whether the adults were reproductively active or not, we paired each male with an adult female on the female web, and checked for courtship behavior (Table 1). We removed the male from the web when it unfolded its pedipalps to the female. These sexually receptive females and males were chosen for the experiments. Egg-holding mothers were taken as sexually unreceptive females because they always held their egg sac with their chelicerae until the spiderlings hatched; generally, they remained sedentary and would not copulate with males during this phase. We randomly paired 30 females with 30 males until mating occurred. Thus, we also obtained gravid spiders.

We tested the hypotheses that the silk of sexually receptive females was attractive to mate-searching males by using a two-choice arena composed of three quadrates consisting of plastic chambers (upper dimensions: 16.5×11 cm; lower: 14.5×9 cm, height: 5.5 cm), each of which component was removable (Fig. 1). A hole was cut into one side of each choice chamber, and in both ends of the release chamber. A selecting male was released into the central chamber and could move freely into the left or right choice chamber.

We completed three sets of trials. The silk of sexually receptive females was presented in all sets, while either silk from juvenile spiders, silk from mate-searching males, or silk from egg-holding females was presented as the alternative choice. Male *P. beijingensis* were introduced into the central release chamber and allowed to acclimate to the surroundings for 1 h before the trials. The silk stimuli consisted of empty webs (no spider present) woven by test spiders. The order of treatments in the arena system was randomized. All male attraction tests were completed at night (usually from 20:00 to 22:00 h). We observed movement of the selecting males under infrared light, and recorded which chamber it first chose during a 2 h observation period. We deleted trials when a selecting male was disturbed and escaped into a choice chamber as soon as the choice arena system was connected. Each selecting male was used in only one trial of one test. A minimum of 15 males was tested in each trial set.

We also tested the attractiveness of extracts of silk from females. Pheromone from the silk of sexually receptive females was extracted with dichloromethane (5 µl for each web). A piece of filter paper containing the extract was placed in one of the choice chambers; the other choice chamber contained a filter-paper with an equal quantity of dichloromethane only. The choice chambers were occupied alternatively with the silk extract and the solvent only. Selecting males were allowed 2 h to discriminate between the silk extract and the solvent treatments.

Table 1 Sequence of courtship behavioral patterns displayed in the spider, *Pholcus beijingensis*

Behavior pattern	Description
Level 1	**Detection and location (♂)**
Pedipalps waggle	Shaking pedipalps up and down
Abdomen vibration	Shaking abdomen, usually immediately following pedipalps shaking
Adjust position	Picking up legs and shuffling body within same location
Search	Shoot jerky movements on web with frequent direction shifts and then turn towards the female
Level 2	**Signaling and approaching (♂)**
Flex	Slow or quick push on web with walking legs while raising body, followed by leg retraction with body lowering to make vibration of the web
Body tremor	High frequency dithering on the web to cause shoot and rapid quiver of the web during walk
Approach	Walk towards female spider with a few interruption for rubbing walking legs with his chelicerae
Pluck web	Short pull on silk threads with one of front legs alternatively in front of the female till she reacts (e.g. plucking back, adjusting direction) alternately, with rarely touching the female legs
Level 3	**Preparation and mate initiation (♂ & ♀)**
Leg spread	Male faces female from a distance of < 2 cm, with femurs of all walking legs spread backward in a line, forming a right angle with the web
Pedipalp unfolding	Male unfolds pedipalps up and forwards with abdomen cocked and legs spread backward, keeping this posture and waiting for approaching female
Contact	Female approaches male, both female and male contact each other with front part of cephalothorax or first pair of walking legs and frequently adjust body position
Mate initiation	Male clamps female's epigynum with his chelicerae and his two pedipalps inserts into the female's genital pore, at the same time female raises all her walking legs together to support her abdomen.

Levels refer to temporal stages within the courtship sequence from transition analysis of *Agelenopsis aperta* presented by Singer (Singer et al. 2000) and *Pholcus beijingensis* presented by Chen (Chen and Li 2005).

Silk Sample Collection and Extraction

Sexually receptive females, mate-searching males, and sexually unreceptive female spiders (egg-holding females) supplied silk samples. Square boxes made of cardboard (22 cm l.×22 cm w.×8.5 cm h.) were used for the spiders to weave webs. A glass Petri dish with cotton soaked in distilled water was set in each box to supply humidity. All boxes were cleaned with alcohol (99%) and air-dried before use. We released the spiders into the boxes individually. After 48 h, the web silk was curled into a very small ball and put into a glass capillary (1.8 mm i.d.×3 cm l.), which had been fused at one end. The capillary containing silk was then sealed in a screw-cap vial (Agilent Technologies, USA). Compounds from each silk sample were extracted with 5 μl dichloromethane (purity > 99.5%, Beijing Fine Chemical Company, Ltd., Beijing, China) for 48 h. Following extraction, we removed the silk, and stored the remaining solution at −20°C until analysis by gas chromatography-mass spectrometry (GC-MS).

Fig. 1 Two-choice arena system used in assessment of the attraction of the silk and the potential pheromones. The three chambers were upended and spliced with a hole on one or two sides. The selecting male was released in the central chamber and could move freely to the left or right choice chamber

GC-MS Analysis

Analytical GC-MS was performed on an Agilent Technologies Network 6890N GC system coupled with 5973 Mass Selective Detector with the NIST/EPA/NIH Mass Spectral Library (2002 version; Agilent Technologies 2002). Chemstation software (Windows 2000) was used for data acquisition and processing. The GC was equipped with a 30 m HP5-MS capillary column (0.25 mm i.d.×0.25 μm film thickness). Helium was used as the carrier gas at a flow rate of 1.0 ml/min. The temperature of the injector was set at 280°C. One μl of sample was injected in the splitless mode. The oven temperature was programmed from 80°C to 240°C at 5°C/min. Then, the temperature was increased by 10°C/min up to 280°C and held for 10 min. Electron impact ionization used 70 eV, and the scanning mass ranged from 30 to 450 amu. Compounds were identified tentatively by matching their gas chromatographic retention times and mass spectra with authentic analogs of the mass spectral library. (*E,E*)-Farnesyl acetate (FA, 95%; Sigma-Aldrich, Inc., St. Louis, MO, USA), diisobutyl phthalate (DIBP, 99.0%; Laboratories of Dr. Ehrenstorfer GmbH, Germany) and hexadecyl acetate (HA, 95%; Sigma-Aldrich, Inc., St. Louis, MO, USA) were used to confirm identification of unknown products after separation on a non-polar column (HP5-MS) and a polar column (HP-INNOWax, 30 m long, 0.25 mm i.d. × 0.25 μm film thickness).

Statistical Analysis

All statistical analyses were conducted using SPSS for Windows (version 15.0; SPSS Inc. 1999). We formed two hypotheses to test statistically. The first stated that no sexual dimorphism occurred in the relative abundances of crude extract compounds from webs. The second stated that there was no difference in the relative abundances of the compounds in the crude extracts from receptive females and sexually unreceptive females. To test the hypotheses, we measured the relative abundance of each compound by converting the peak area of a particular compound into a percentage of the sum peak areas from the 14 main GC peaks. If a given GC peak was too small to display the diagnostic MS ions, which rarely occurred, its area was taken as zero. Subsequently, the relative abundances of the compounds were analyzed by using either parametric tests, when the data were normally distributed, or non-parametric tests, when the data were not normally distributed. To analyze differences in the relative abundances of the compounds, we used Mann–Whitney *U* test for compound 8 when comparing differences between sexually receptive females and mate-searching males, and compound 14 in comparing differences between sexually receptive females and egg-holding females, which did not have normally distributed raw data. Other compounds were analyzed with an independent two-tailed *t* test when the raw data were normally distributed. The Chi-square test was used to analyze observed counts to the expected counts for male choice data.

Titer Analysis of Putative Pheromone Components

We determined the content of each putative pheromone component on a female's web. FA, HA, and DIBP each were diluted sequentially in dichloromethane to concentrations of 0.0001, 0.001, 0.01, 0.1, and 1 μg/μl. We injected 1 μl of each prepared solution into the GC-MS. GC detection showed that the peak areas of these compounds in female silk extract were close to those of the test samples at 0.01 μg/μl. Therefore, we injected 1, 2, 3, 4, and 5 μl of authentic sample at 0.01 μg/μl in GC to obtain the calibration regression equation. The quantity of each putative pheromone component on a female's web was calculated by comparing the peak area with that of the synthetic standard sample.

Test for Biological Activity of the Potential Pheromone Components

Bioassay of the potential pheromone components involved three steps. First, attractiveness of each compound to mate-

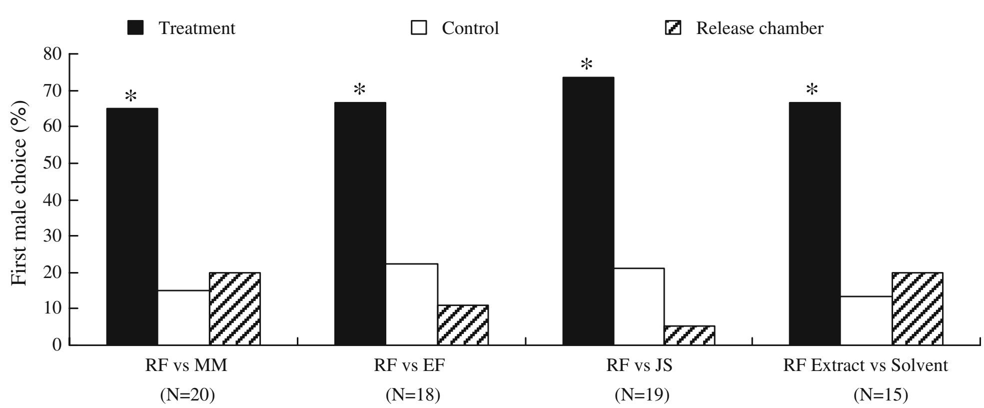

Fig. 2 Results of male *Pholcus beijingensis* attraction to sexually receptive female silk and the silk extract. Trials were completed in the two-choice arena system (Fig. 1). *RF* web of the sexually receptive female; *MM* web of the mate-searching male; *EF* web of the egg-holding female; *JS* web of the juvenile spider; *RF Extract* silk extract of the sexually receptive female; *Solvent* an equivalent amount of dichloromethane to the silk extract. The catalogue 'treatment' refers to sexually receptive female webs in all trials; 'Control' refers to webs of other spiders including mate-researching male, egg-holding female, and juvenile spider in corresponding trials; 'Release chamber' refers to males that within the 2 h observation period failed to leave the central arena into which they had been introduced 1 h before the start of the trial. * indicates $P < 0.05$

searching males was tested in the two-choice arena. Second, attractiveness of the tertiary blend involving FA, HA, and DIBP was tested. Third, we tested binary blends to check whether attractiveness to males was retained as follows: FA and DIBP; FA and HA; and HA and DIBP. The relative quantity of each component in all trials was equivalent to that naturally contained in a female's web.

Because the blend of FA and HA was significantly attractive to males, we diluted FA and HA in dichloromethane to concentrations of 0.001, 0.01, 0.1, and 1 μg/μl, and we used 10 μl FA and 5 μl HA of the series to determine the attractiveness of differing concentrations. An equivalent amount of dichloromethane was applied as the solvent control. We observed the movement of the males under infrared light for 2 h, and recorded which chamber they chose first.

We also determined whether the pheromone would elicit male courtship. We placed a filter-paper with the pheromone (10 μl FA and 5 μl HA at a concentration of 0.01 μg/μl) on an empty egg-holding female web, and then introduced a male. We observed each male for 2 h to detect whether the male displayed courtship signaling or not. We also observed male mate-searching behavior on empty webs produced by sexually receptive females.

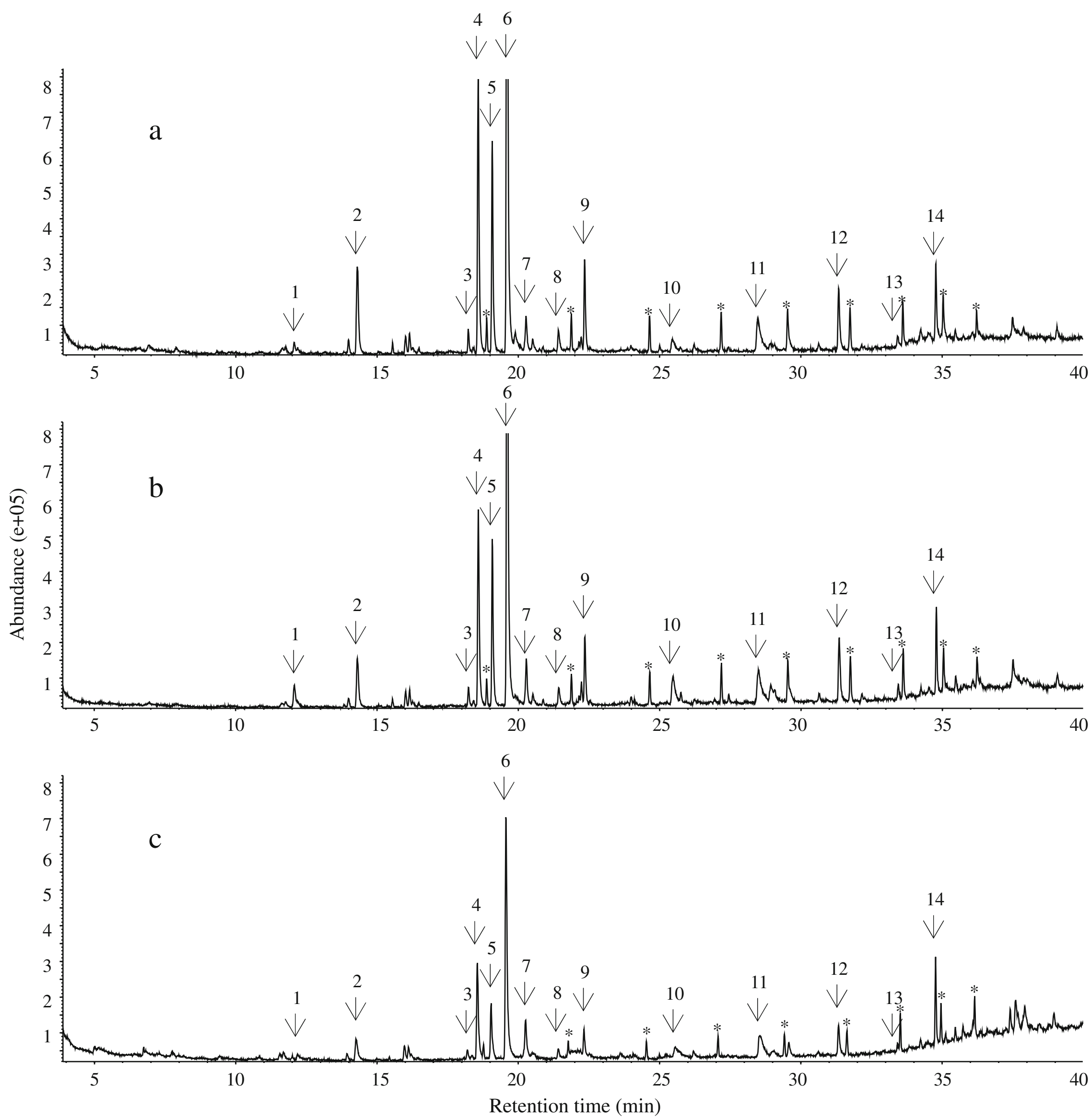

Fig. 3 Representative gas chromatograms of the crude extract of sexually receptive female silk **a**, mate-searching male silk **b** and egg-holding female **c**. The numbers that label the GC peaks correspond to peak numbers in Table 2. * denotes the Si-containing compounds that are regularly presented in the chromatograms. These silaceous compounds were not considered as potential pheromone components but contaminants from the GC column

Results

Male Attraction to Sexually Receptive Females' Silk and Silk Extract

The results of the two-choice arena trials are presented in Fig. 2. Adult male *P. beijingensis* were significantly attracted to webs produced by sexually receptive females (Chi-square test: $X_1^2=6.250$, $P<0.05$; $X_1^2=4.000$, $P<0.05$; $X_1^2=5.556$, $P<0.05$, respectively; Fig. 2). Dichloromethane extract of the silk from sexually receptive females also attracted mate-searching males ($X_1^2=5.333$, $P<0.05$; Fig. 2). The selecting males moved from the release chamber directly into the chamber previously occupied by sexually receptive females, and most males would stay on the empty females' webs until the next morning.

Identification of Potential Pheromone Components

More than 20 different compounds were regularly detected (some in low quantities) in silk samples of *P. beijingensis*, including some silaceous compounds. These were present in all samples and are considered most likely to be contaminants from the column; therefore, they were dismissed (Fig. 3).

We tentatively identified 14 compounds detected in the silk samples by matching GC retention times and mass spectra with analogs in the mass spectral library (Table 2). No qualitative differences were observed in chromatograph peaks among the silk extracts of the sexually receptive and egg-holding females and the mate-searching males. Egg-holding females usually spun less silk than sexually receptive females and mate-searching males. The total peak area detected from the silk extract of egg-holding females was less than those obtained from the other sample groups.

Quantitative analyses of relative abundances of the relevant compounds are presented in Table 2. Relative areas of peaks 5, 6, and 9 occurred in significantly greater proportions in silk extracts of the sexually receptive females than those of mate-searching males. These compounds also displayed significant differences between receptive females and egg-holding females, and they were less abundant in the silk extract of egg-holding females (Table 2). Therefore, they were considered as putative pheromone components of *P. beijingensis*. The mass spectra (Fig. 4) matched those of synthetic standards following separation with non-polar and polar columns. Thus, compounds 5, 6, and 9 were (*E,E*)-farnesyl acetate (FA), diisobutyl phthalate (DIBP), and hexadecyl acetate (HA), respectively.

Table 2 Comparison of relative abundance of compounds in silk extract of the spider *Pholcus beijingensis* (Mean ± *SD*)

Peak No.	Retention Time (min)	Compounds	Relative Abundance (%)			Statistical Significance (*P*)	
			RF (*N*=8)	MM (*N*=8)	EF (*N*=9)	RF *vs* MM	RF *vs* EF
1	12.06	Butylated hydroxytoluene	0.44±0.25	2.01±0.92	0.72±0.71	0.002	0.301
2	14.31	Tetradecanal	3.30±1.99	4.58±1.13	5.98±1.51	0.136	0.007
3	18.23	Octadecane	0.98±0.33	0.91±0.27	1.25±0.39	0.633	0.140
4	18.58	Hexadecanal	9.11±4.77	12.25±2.88	16.20±4.72	0.133	0.008
5[c]	19.08	*E,E*-farnesyl acetate [a]	11.28±2.91	8.02±1.11	1.74±1.36	0.010	0.000
6[c]	19.60	Diisobutyl phthalate [a]	44.65±11.66	32.73±3.74	10.56±6.12	0.024	0.000
7	20.28	2-heptadecanone	1.85±0.89	2.89±0.67	4.93±1.58	0.020	0.000
8	21.43	Dibutyl phthalate	1.68±0.20	1.54±0.21	1.77±0.66	0.065 [b]	0.718
9[c]	22.36	Hexadecyl acetate [a]	4.89±1.10	3.48±0.34	1.30±1.22	0.004	0.000
10	25.46	Hexadecanamide	1.19±0.96	6.46±2.83	5.53±3.47	0.001	0.005
11	28.48	Oleyl amide	7.02±5.13	9.64±2.90	15.86±5.72	0.230	0.005
12	31.34	Diisooctyl phthalate	5.48±0.81	5.40±0.47	5.75±1.89	0.809	0.703
13	33.42	Unidentified	0.86±0.61	2.11±1.09	3.23±2.85	0.014	0.039
14	34.78	Squalene	7.25±4.06	7.96±2.93	25.19±13.51	0.693	0.002 [b]

RF Sexually receptive female; *MM* Mate-searching male; *EF* Egg-holding female

[a] Compounds were verified with synthetic standard samples after separation with a non-polar column and a polar column; other components were identified tentatively by comparison with spectra listed in the NIST (Agilent Technologies 2002) mass spectral library and analogous data except for the unidentified compounds, whose mass spectra has low matching degree with those suggested by the MS library (NIST 2002)

[b] *P* values were tested by using Mann–Whitney U test; others were tested by using independent *T* test

[c] Relative abundances of these components in silk extract of sexually receptive females was significantly more than those in silk extracts of mate-searching males and egg-holding females

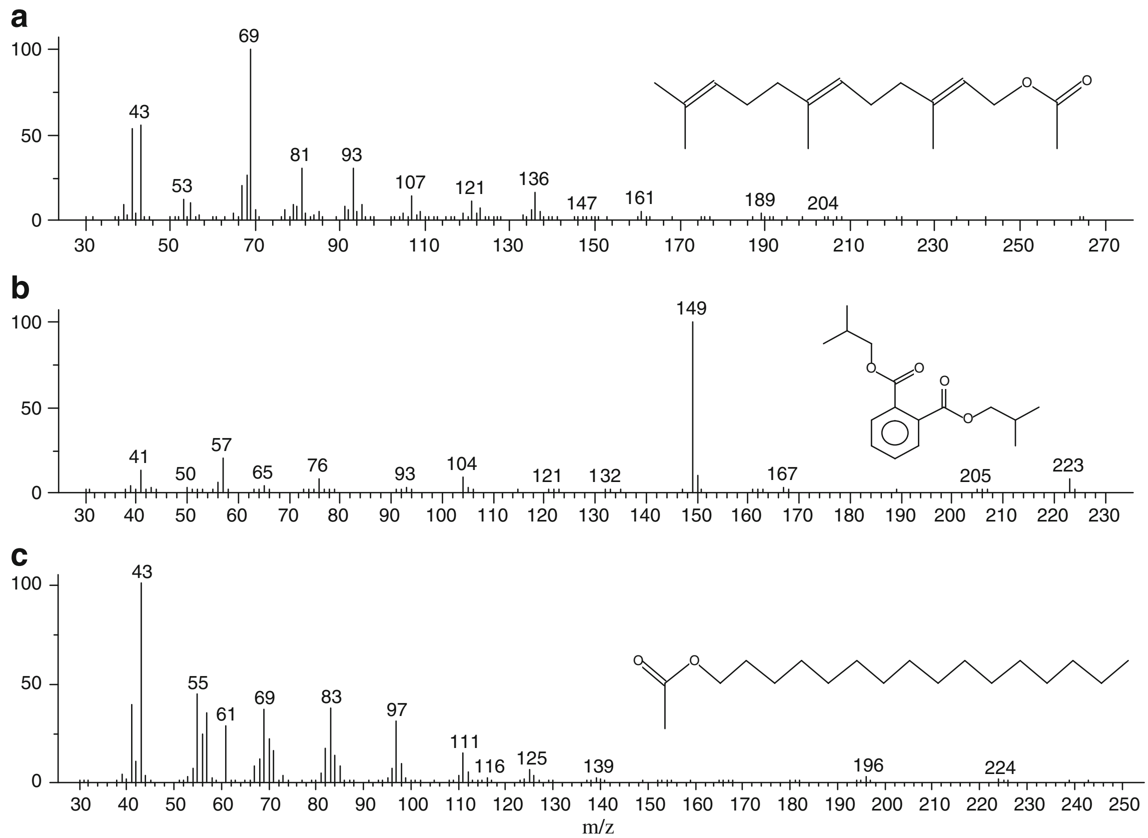

Fig. 4 Mass spectra of **a** compound 5, **b** compound 6, and **c** compound 9 of the silk sample. They were identified as (*E,E*)-farnesyl acetate, diisobutyl phthalate, and hexadecyl acetate, respectively, by comparing retention times and mass spectra with those of the authentic standards

Titer Analysis of the Putative Pheromone Components

The quantities of FA and HA on female webs (N=8) was determined by external regression analysis of concentration ranges for synthetic standards of these compounds. Sexually receptive females produce about 0.052 and 0.025 µg/web of FA and HA, respectively.

Biological Activity of the Potential Pheromones

Bioassay results showed that male *P. beijingensis* displayed no choice for a particular chamber in the trials involving the single compounds FA, DIBP, or HA (X_1^2=0.529, P>0.05; X_1^2=0.222, P>0.05; X_1^2=0.474, P>0.05, respectively; Fig. 5). However, when we combined the three compounds, the tertiary blend of FA, HA, and DIBP was attractive to selecting males (X_1^2=4.263, P<0.05; Fig. 5). The binary blend of FA and HA also showed significant attraction to males (X_1^2=4.000, P<0.05; Fig. 5), while the other two binary blends did not attract males (X_1^2=0.053, P>0.05; X_1^2=0.818, P>0.05) ; Fig. 5). Whereas FA and HA were required to attract males, DIBP was not, even if it occurred in a large amount in the silk. As the silk samples were collected from the cardboard boxes with a slice of plastic paper filmed in the inner wall, we cut down a small chip of the plastic paper (3×3 mm) and extracted it with dichloromethane. GC-MS detection result showed that the phthalate compounds including diisobutyl phthalate, dibutyl phthalate, and diisooctyl phthalate were presented in the extraction. Therefore, the phthalates must be contaminants introduced on the webs by the spiders coming in contact with the plastic.

Attractiveness of the binary blend of FA and HA in different concentrations was tested (Fig. 6). Male *P. beijingensis* significantly preferred the chamber containing FA and HA at concentrations of 0.01 and 0.1 µg/µl (X_1^2=4.571, P<0.05; X_1^2=7.118, P<0.05, respectively; Fig. 6). Males showed no significant choice between the treatment chamber and control chamber in the trials involving the binary blend at concentration of 0.001 µg/µl and the highest concentration of 1 µg/µl (X_1^2=1.143, P>0.05; X_1^2=2.579, P>0.05; Fig. 6).

Most males displayed exploratory behavior (Table 1, Level 1) but no males displayed courtship signaling (Table 1, Levels 2 and 3) when presented with pheromone solutions on filter-paper on the webs made by egg-holding females. Likewise, only searching behavior, and no courtship signaling, was exhibited by males on the webs produced by sexually

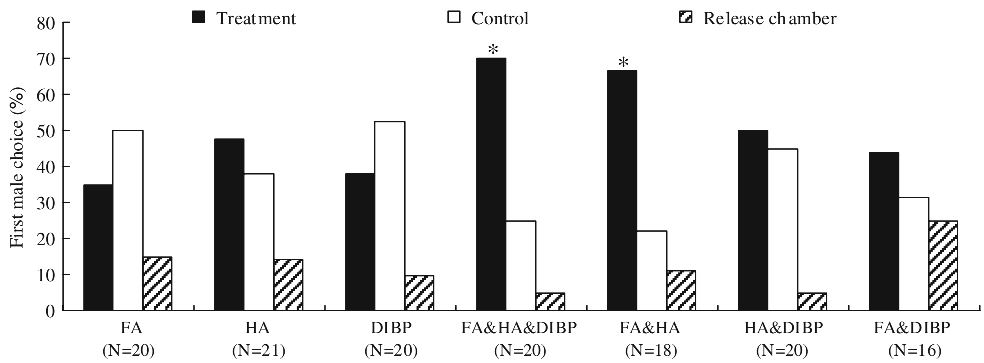

Fig. 5 Results of the attractiveness for male *Pholcus beijingensis* to single putative pheromone component and the blends. Trials were completed in the two-choice arena system (Fig. 1). Dosage of the chemicals applied in the treatments was equal to those obtained from one female web. 'Control' means equal quantity dichloromethane to corresponding applied chemicals. 'Release chamber' refers to males that within the 2 h observation period failed to leave the central chamber into which they had been introduced 1 h before the start of the trial. * indicates $P < 0.05$

receptive females. Male *P. beijingensis* initiated signaling only when a sexually receptive female was on the web.

Discussion

The sex pheromone of *P. beijingensis* consists of a 2:1 ratio of (*E,E*)-farnesyl acetate (FA) and hexadecyl acetate (HA), deposited on the web by sexually receptive females. By triggering male searching behavior, the pheromone plays an important role in guiding males to females' webs. Male *P. beijingensis* showed no attraction response to either FA or HA alone. Although both females and males of *P. beijingensis* can emit the pheromone, sexually receptive females release much more than males. FA is relatively abundant on the female's silk, averaging 0.052 µg per web, while HA averages 0.025 µg per web. Mixtures of FA and HA at or below 0.01 µg and 0.005 µg, respectively, failed to attract selecting males. Thus, male *P. beijingensis* have an 'olfactory detection threshold' for finding potential mates. Pheromone in a slightly higher concentration than that generally found on the web of a sexually receptive female attracted males and stimulated mate-searching behavior (Fig. 6). However, males were not attracted when the pheromone dosage was much higher than that of a sexually receptive female's web.

In other species of spiders, however, single pheromone compounds bound to the silk act as sex attractants (Papke et al. 2000, 2001; Tichy et al. 2001). The first reported spider sex pheromone, that of *Linyphia triangularis*, comprised two compounds, (*R*)-3-hydroxybutyric acid (HBA) and (*R*)-3-[(*R*)-3-hydroxybutyryloxy]-butyric acid (HBBA), and either compound alone could trigger the web reduction behavior of males. The dimer (HBBA) and the monomer (HBA) are relatively unstable compounds, such that HBBA slowly disintegrates into the monomer, HBA. Thus, HBBA

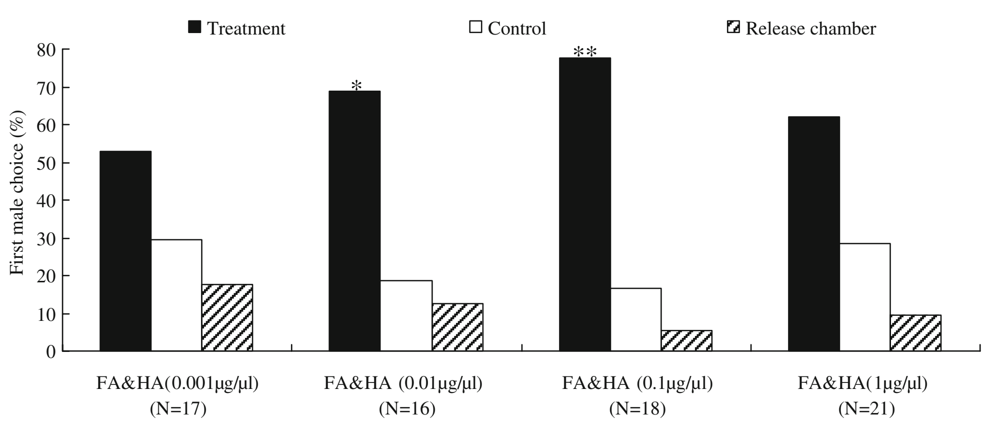

Fig. 6 Results of the attractiveness for male *Pholcus beijingensis* to four concentrations of the two-component blend and each component of the blend. Trials were completed in the two-choice arena system. FA is (*E,E*)-farnesyl acetate and HA is hexadecyl acetate. The category 'treatment' refers to one of the stepwise concentrations of the binary blend. 'Control' refers to equivalent dichloromethane to treatment. 'Release chamber' refers to males that within the 2 h observation period failed to leave the central arena into which they had been introduced 1 h prior to the start of the trial. * indicates $P < 0.05$. ** indicates $P < 0.01$

may be the precursor of HBA (Schulz and Toft 1993). (*R*)-HBA occurs in three closely related genera of spiders: *Linyphia*, *Microlinyphia*, and *Neriene* (Schulz and Toft 1993).

The major pheromone component of *P. beijingensis* (FA), was identified previously as a sex pheromone component of the click beetle, *Agriotes proximus* (Coleoptera: Elateridae) (Yatsynin et al. 1980, 1996). This compound also was reported from male Scandinavian bumblebees, *Bombus pratorum* (Bergman and Bergström 1997), the rock honeybee *Apis dorsata* (Blum et al. 2000), and the stingless bee *Melipona beecheii* (Cruz-López et al. 2005). Recently, FA was described as a sex pheromone component in the preputial gland of male Brandt's voles, *Lasiopodomys brandtii*, where it attracts females (Zhang et al. 2007b). HA also was described as a pheromone component in voles (Brinck and Hoffmeyer 1984; Welsh et al. 1988; Zhang et al. 2007a). Straight-chain acetates usually containing 12–18 carbons are common pheromone components in other insects (Roelofs 1995; Byers 2002).

Most insect sex pheromones are blends of chemicals that consist of a number of different components, and they function only when combined in a particular ratio (Wyatt 2003). Multicomponent pheromones also occur in vertebrates, such as goldfish, mice and other mammals; here too, individual components of the blend usually are inactive (Novotny et al. 1999; Sorensen and Stacey 1999). It is not uncommon that some pheromone components are used by several, distantly related species. The Asian elephant, *Elephas maximus*, shares its female sex pheromone with 140 species of moth (Rasmussen et al. 1996). Zhang et al. (2007b) reported that most of the castration-suppressed compounds in preputial gland secretions of male Brandt's voles were described previously as pheromone components of insects. The sex pheromone compounds from the spider *L. triangularis* (HBA and its dimer HBBA) also have been found in an ascomycete fungus, *Hypoxylon truncatum* (Quang et al. 2003). The coincidence of pheromone components among different species illustrates not only the ubiquity of pheromones, but also their biochemical convergence (Wyatt 2003).

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