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Absolute configuration, improved synthesis and femtogram-level behavioral activity of the sex pheromone of the minute parasitoid wasp *Trichogramma turkestanica*

Teris A. van Beek[1]✉, Jeya P. Kaniraj[2], Alexander Dornbusch[3], Hans M. Smid[4], Mark Houtman[2], Konstantin Czak[3], Hans Beijleveld[1], Isabel M. M. S. Silva[4], Maarten A. Posthumus[1], Joop J. A. van Loon[4], Nina E. Fatouros[5], Wittko Francke[6], Adriaan J. Minnaard[2]✉ & Joachim Ruther[3]✉

Hymenopteran egg parasitoids of the genus *Trichogramma* are among the tiniest insects on Earth (< 0.5 mm in size, 8 µg) and are important biological control agents of moth pests. The existence of sex pheromones has been demonstrated in *Trichogramma* species, but none has yet been fully characterized. Here, we report absolute configuration, improved synthesis and behavioral activity of the sex pheromone of *T. turkestanica*, making it one of the smallest insects with a fully characterized sex pheromone. Enantioselective synthesis and chiral gas chromatography were used to determine the absolute configuration of the previously identified, stereochemically complex dienol (2*E*,4*E*,6*S*,8*S*,10*S*)-4,6,8,10-tetramethyltrideca-2,4-dien-1-ol and to newly establish the same stereochemistry for the corresponding diene (2*E*,4*E*,6*S*,8*S*,10*S*)-4,6,8,10-tetramethyltrideca-2,4-diene. In a 3:1 mixture of dienol and diene applied to two solvent-washed wasps (dummies), these compounds triggered attraction, arrestment, and courtship ("casting") in males at doses, as little as 670 attograms per dummy. This pheromone could be studied to monitor *T. turkestanica* field populations, with the aim to improve the efficiency of biocontrol strategies.

Egg parasitoids, including those of the genus *Trichogramma* (Hymenoptera, Trichogrammatidae), are among the smallest insects on Earth, measuring less than 0.5 mm in size. With more than 200 species, *Trichogramma* wasps are economically important biological control agents used worldwide in the field, in greenhouses, warehouses as well as in private households[1–3]. Females lay their eggs into lepidopteran (butterflies and moths) host eggs, and the developing wasp larvae kill and consume the host embryo[4]. *Trichogramma* wasps are commercially available and typically provided in batches of up to 200,000 parasitized host eggs, from which the egg parasitoids emerge followed by mating and dispersal in the search for new host patches.

As with most parasitoids, the chemical sense of *Trichogramma* wasps is crucial for both host and mate finding[5,6]. Their antennae, the major sites of perception of volatile semiochemicals such as pheromones, exhibit a distinct sexual dimorphism with males possessing conspicuously long olfactory sensilla lacking in females (Fig. 1)[7,8]. This suggests the involvement of female-derived sex pheromones in sexual communication.

[1]Laboratory of Organic Chemistry, Wageningen University, Stippeneng 4, 6708 WE Wageningen, Netherlands. [2]Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 7, 9747 AG Groningen, Netherlands. [3]Institute of Zoology, University of Regensburg, Universitätsstraße 31, 93053 Regensburg, Germany. [4]Laboratory of Entomology, Droevendaalsesteeg 1, 6708 PB Wageningen, Netherlands. [5]Biosystematics Group, Droevendaalsesteeg 1, 6708 PB Wageningen, Netherlands. [6]Institute of Organic Chemistry, University of Hamburg, Mittelweg 177, 20148 Hamburg, Germany. ✉email: teris.vanbeek@wur.nl; a.j.minnaard@rug.nl; joachim.ruther@ur.de

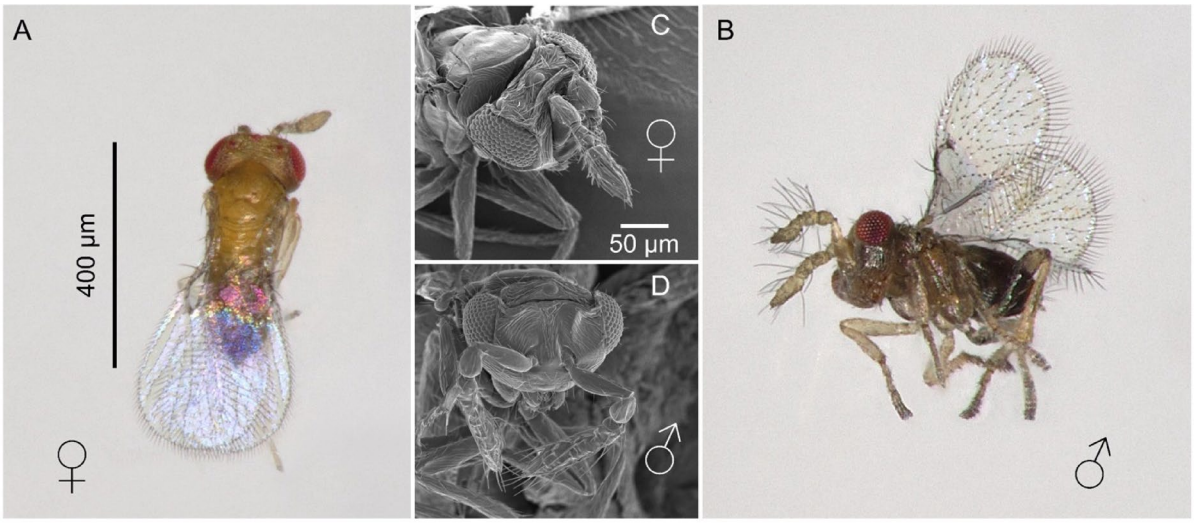


Fig. 1. Sexual dimorphism in *Trichogramma turkestanica*. Images of (**A**) female, (**B**) male *T. turkestanica* wasps, (**C**,**D**) close-up images of female and male head obtained by scanning electron microscopy (SEM) showing the sexual dimorphism of olfactory sensilla. Size bars of (**A**) and (**C**) apply also for (**B**) and (**D**), respectively.

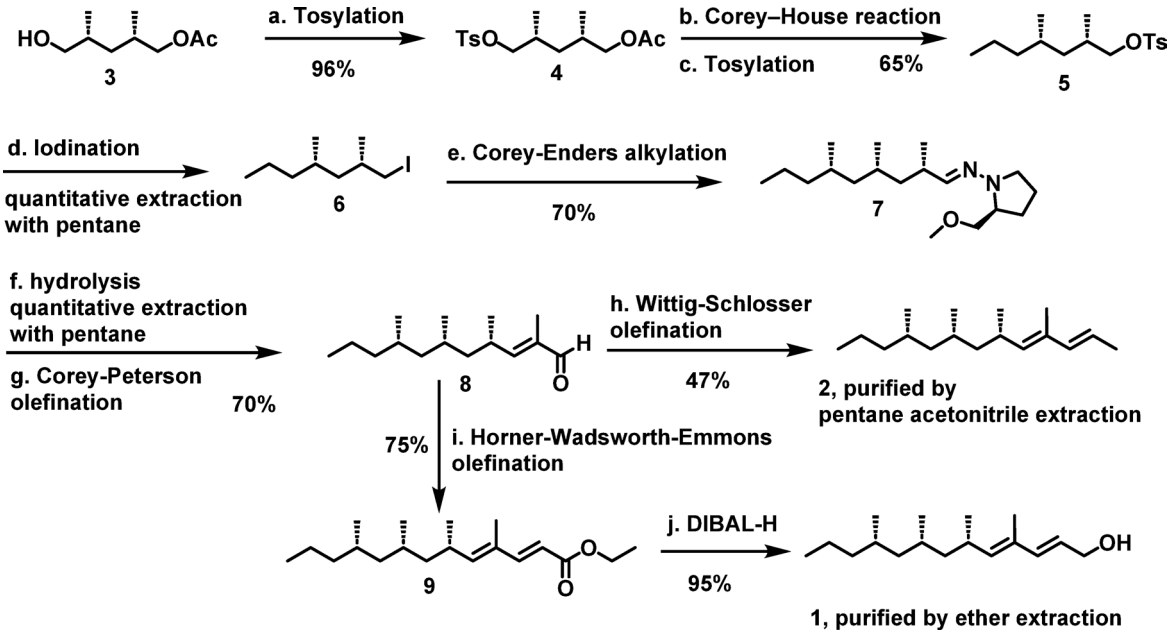
While behavioral studies have demonstrated the existence of female pheromones in several *Trichogramma* species[6,9–12], not a single *Trichogramma* pheromone has yet been fully characterized. Adult *Trichogramma* wasps are barely visible to the naked eye and weigh no more than 8 µg[13]. Thus, pheromone release is extremely low, i.e., in the low picogram per hour range[14]. This impedes the use of common structure elucidation techniques such as NMR spectroscopy. This, together with the often non-scalable production of virgin female wasps, makes structure elucidation of sex pheromones of insects smaller than 1 mm such as *Trichogramma* wasps a daunting task.

Previously, we reported on the partial elucidation of the putative sex pheromone of *Trichogramma turkestanica*[13–16]. In short: when virgin females were sampled by solid phase microextraction (SPME), two compounds, a hydrocarbon (MW = 236 Da) and the corresponding alcohol (MW = 252 Da), could be consistently detected by coupled gas chromatography-mass spectrometry (GC–MS). These compounds were neither produced by males nor by males and females together. It was thus hypothesized that these two compound might be sex pheromones. The total amount extracted from a SPME fiber after sampling 50–100 virgin females during 20–48 h for both compounds combined was determined at approximately 4 ng[14]. Based on interpretation of high-resolution mass spectra, retention indices on two columns, two derivatizations at the low ng scale followed by GC–MS, and biosynthetic considerations[17], 2,6,8,12-tetramethyltrideca-2,4-diene and the corresponding primary allylic alcohol were proposed as structures[14]. Synthesis of a library of isomers of the diene showed that their MS was very similar but not identical to that of the pheromone[15]. Subsequent synthesis and non-chiral GC–MS analysis of a mixture of all 16 isomers of (2*E*,4*E*/*Z*)-4,6,8,10-tetramethyltrideca-2,4-diene **2** showed that seven out of eight racemic stereoisomers present could be separated. The natural product coeluted with the first eluting peak of the 4*E*-series and mass spectra were identical. Based on biogenetic principles[17], racemic 2*E*,4*E*/*Z*,6*S*,8*S*,10*S*-tetramethyltrideca-2,4-diene **2** (*syn*,*syn* configuration, see Scheme 1) was synthesized next as most likely isomer[15]. The later eluting 4*E* isomer indeed showed the same retention time on three different GC columns as the natural product. GC–MS analysis of a synthetic mixture of all 16 possible (2*E*,4*E*/*Z*) isomers, the four 2*E*,4*E*/*Z* *syn*,*syn* isomers and enantiopure **1** (see Scheme 1), proved the carbon skeleton and relative configuration of the later eluting alcohol[15,16]. Chiral separation of racemic **1** and **2** was attempted at 100 °C on a heptakis-[2,3-di-*O*-methyl-6-*O*-(*tert*-butyldimethylsilyl)]-β-cyclodextrin (50% in OV-1701) but was unsuccessful[16].

Thus, the absolute configuration of the two hypothetical sex pheromone components remained unknown as well as their putative bioactivity. For this purpose also enantiopure **2** was required as well as a new synthesis of **1**, if the natural product possessed the 6*R*,8*R*,10*R* stereochemistry. Thus, the aim of this study was: (1) to determine the absolute configuration of **1** and **2**; (2) an improved enantioselective synthesis of both compounds; (3) to prove through bioassays that one or both putative components are constituents of the sex pheromone of *T. turkestanica*. Herein we report the absolute configuration of the components of this pheromone based on chiral GC–MS and asymmetric synthesis. Behavioral experiments demonstrate biological activity of the synthetic pheromone candidates and their extreme potency, making *T. turkestanica* the smallest insects whose sex pheromone has been fully elucidated.

Results and discussion

First an alternative chiral stationary phase ([2,3,6-tri-O-methyl]-β-cyclodextrin (20% in 35% phenyl/65% dimethylsiloxane)) was evaluated. This phase in combination with a slow temperature gradient from 105 to



Scheme 1. Synthesis of 1 and 2 via enzymatic desymmetrization and stereoselective alkylation[a]. [a]Reaction conditions: Synthesis of **1** and **2**. Reagents and conditions: (a) TsCl, pyridine (b) CuBr·SMe_2, EtMgBr, THF (c) TsCl, pyridine (d) NaI, CH_3CN, 95 °C (e) 2,2,6,6-tetramethylpiperidine, BuLi, 0 °C, then (*S*)-(−)-1-amino-2-(methoxymethyl)pyrrolidinyl propan-1-imine, −78 °C, then **6**, THF (f) 3 M HCl in water/pentane (g) *N*-cyclohexyl-2-(triethylsilyl)propan-1-imine, *s*-BuLi, −78 °C, then 2,4,6-trimethyl nonanal, −20 °C, THF (h) ethyltriphenylphosphonium bromide, LiBr, PhLi, then **8**, then PhLi, *t*-BuOH, THF (i) and (j) see: Geerdink et al.[16]

160 °C at 0.5 °C/min marginally but significantly resolved the two enantiomers of both **1** and **2** (α-values of 1.004 and 1.006 respectively, see Fig. 2D and A). Injection of the available 6*S*,8*S*,10*S*-enantiomer of **1**[16], showed it to be the earlier eluting enantiomer (Fig. 2E) and identical with the natural product (Fig. 2F). Having established the absolute configuration of the alcohol, the focus was shifted to the synthesis of the 6*S*,8*S*,10*S*-enantiomer of **2** as it was assumed to have the same absolute stereochemistry as **1**.

The earlier synthesis of **1** comprised 16 steps with only 4% overall yield[16]. To establish the absolute configuration of **2** and to have sufficient amounts of **1** and **2** for studying their biological activity, a considerably more efficient synthesis giving access to both compounds was developed (Scheme 1)[18,19]. This synthesis commenced with the enantiopure acetate **3**, prepared by enzymatic desymmetrization of the meso-diol[20,21]. After several functional group manipulations and a copper-catalyzed alkylation, a productive Corey-Enders hydrazone alkylation introduced the third methyl-branch. Subsequent hydrolysis and Corey-Peterson olefination produced intermediate **8** which was used for both the preparation of **1** and **2**, building on previously developed methods[22].

Compound **8** is identical to the marine polypropionate siphonarienal and also serves as a key intermediate for several other natural products such as siphonarienone and (−)-lardolure, the aggregation pheromone of the acarid mite *Lardoglyphus komoi*[23]. Care was taken to avoid column chromatography of the volatile intermediates and the acid sensitive end products. An acetonitrile/pentane extraction proved advantageous in the isolation of pure **2**. This synthesis (Scheme 1) produced pure **2** in 8 steps with 14% overall yield and pure **1** in 9 steps with 22% overall yield. Chiral GC confirmed that natural hydrocarbon **2** has, as expected, also the 6*S*,8*S*,10*S* configuration (Fig. 2A–C).

To demonstrate biological activity of the synthetic pheromone candidates, we chose the bioassay set-up used earlier for pheromone studies with parasitoid wasps[24]. In short: it consisted of an illuminated round observation chamber (⌀ 10 mm, 3 mm height) made from acrylic glass ("arena") covered by a cover slip. The arena was subdivided into an outer and inner zone by a circular marking (⌀ 5 mm). Compounds to be tested were applied on dead, dichloromethane-washed and dried female wasps ("dummies") which did not elicit any behavioral responses in males. A sequential dilution series (in methyl *tert*-butyl ether) in 1:10 steps starting with 30 ng/µL of **1** and 10 ng/µL of **2** was prepared (Table S1 in the Supplementary Information). The 3:1 ratio of **1** and **2** was chosen according to the ratio reported previously in the headspace of live virgin females[14]. For each test, groups of 60 dummies were treated with dilutions of **1** and/or **2** (10 µL each) or with 20 µL of the pure solvent (control). After evaporation of the solvent, two treated dummies were placed in the center of the arena, a male was placed at the edge of the arena, and its behavior was observed for 5 min under a stereo microscope. As a proxy for male attraction, it was recorded when the first contact (antennation or direct mounting) with one of the dummies occurred. The time spent by the male on a dummy or inside the inner circle of the arena was recorded as a proxy for male arrestment. Finally, it was recorded whether and how often the male showed casting (video S1 in the Supplementary Information) or copulation attempts. Casting describes conspicuous zigzagging

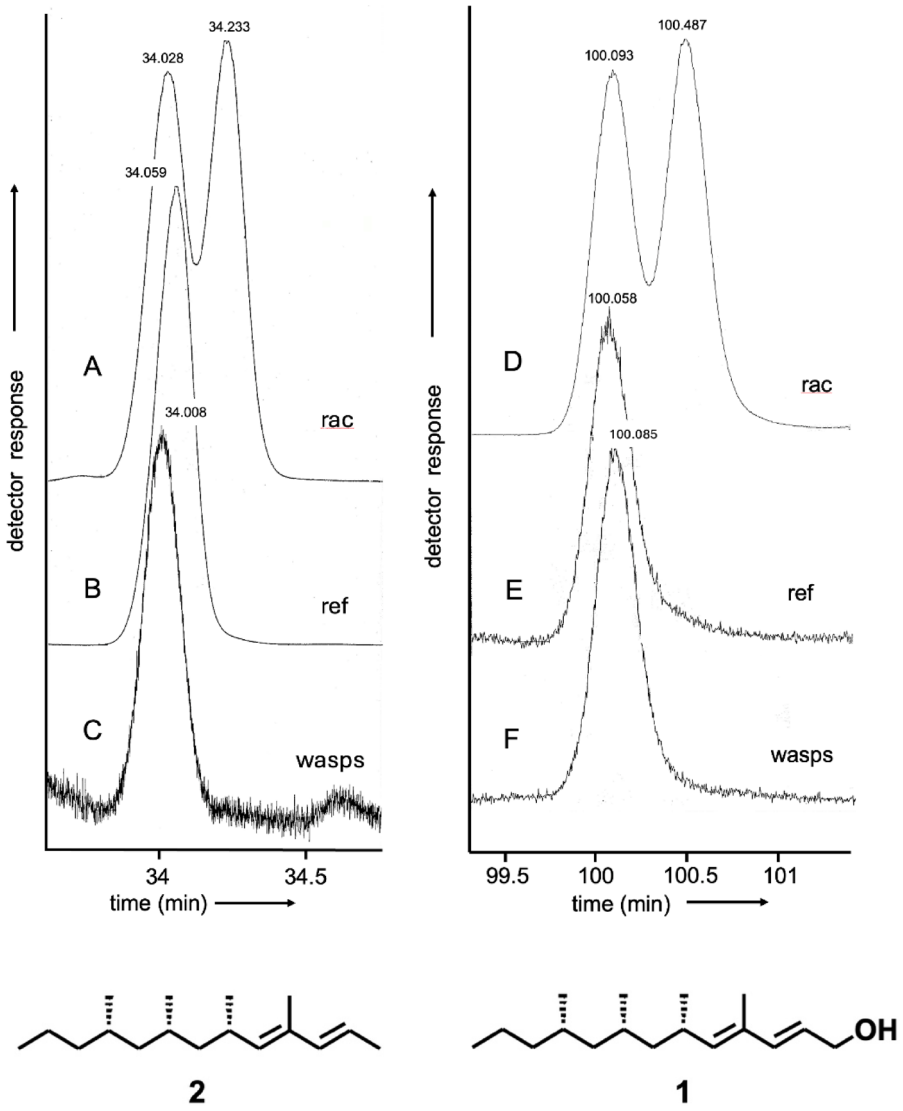


Fig. 2. Enantioselective separation of *T. turkestanica* pheromone candidates. (**A**–**C**) overlay of the chiral gas chromatography-flame ionization detector (GC-FID) profiles of (**A**) synthetic racemic (rac) **2**; (**B**) enantiopure (ref) **2**; (**C**) headspace volatiles from 50 virgin *T. turkestanica* females (wasps) sampled by solid phase microextraction (SPME). (**D**–**F**) overlay of the chiral gas chromatography-mass spectrometry (GC–MS) total ion current profiles of (**D**) synthetic racemic (rac) **1**; (**E**) enantiopure (ref) **1**; (**F**) headspace volatiles from 18 virgin *T. turkestanica* females sampled by SPME (wasps). Below the chromatograms, the corresponding structures of **2** (diene) and **1** (dienol) are given.

movements shown by many insect species when perceiving pheromones and other wind-borne odors[25]. Casting has also been described in *T. turkestanica* males when approaching virgin females and has been suggested to be the first step of male courtship behavior[13,14]. The 3:1 mixture of **1** and **2** was active from 3 + 1 ng/µL to 3 + 1 fg/µL in triggering male casting (Chi² contingency table test: Chi^2=36.206, df = 8, n = 25, p < 0.0001, Fig. 3A, Table S2 in the Supplementary Information) and arrestment (Kruskal-Wallis H = 22.96, df = 8, n = 25, p = 0.0034, Fig. 3B, Table S3 in the Supplementary Information). The same held true for the activity expressed as castings per 5 min (Kruskal-Wallis H = 29.62, df = 8, n = 25, p < 0.0001 Fig. S1 and Table S4 in the Supplementary Information). Males contacted dummies treated with **1** and **2** (range 300 + 100 pg/µL – 300 + 100 fg/µL) significantly faster when compared to solvent-treated control dummies (Fig. 3C). For example, eleven males (44%) contacted at least one of the dummies within the first 60 s at 300 + 100 pg/mL while in the solvent control only two males (8%) did so (Fisher's exact test: Chi^2=6.65, df = 1, p = 0.0083). Both compounds were also behaviorally active when tested alone with respect to both casting (Chi² contingency table test: Chi^2=71.09, df = 9, n = 25, p < 0.0001, Fig. 3D, Table S5 in the Supplementary Information) and arrestment (Kruskal-Wallis test: H = 67.39, df = 9, n = 25, p < 0.0001, Fig. 3E, Table S6 in the Supplementary Information). The lowest active concentration of the dienol **1** when tested alone was 30 pg/µL (casting) and 0.3 pg/µL (arrestment), respectively. The diene **2** alone was active at 1 ng/µL but no longer when further diluted. This indicates that **1** is the primary contributor to the attractiveness of the pheromone and that its activity is enhanced by **2**, particularly at low concentrations.

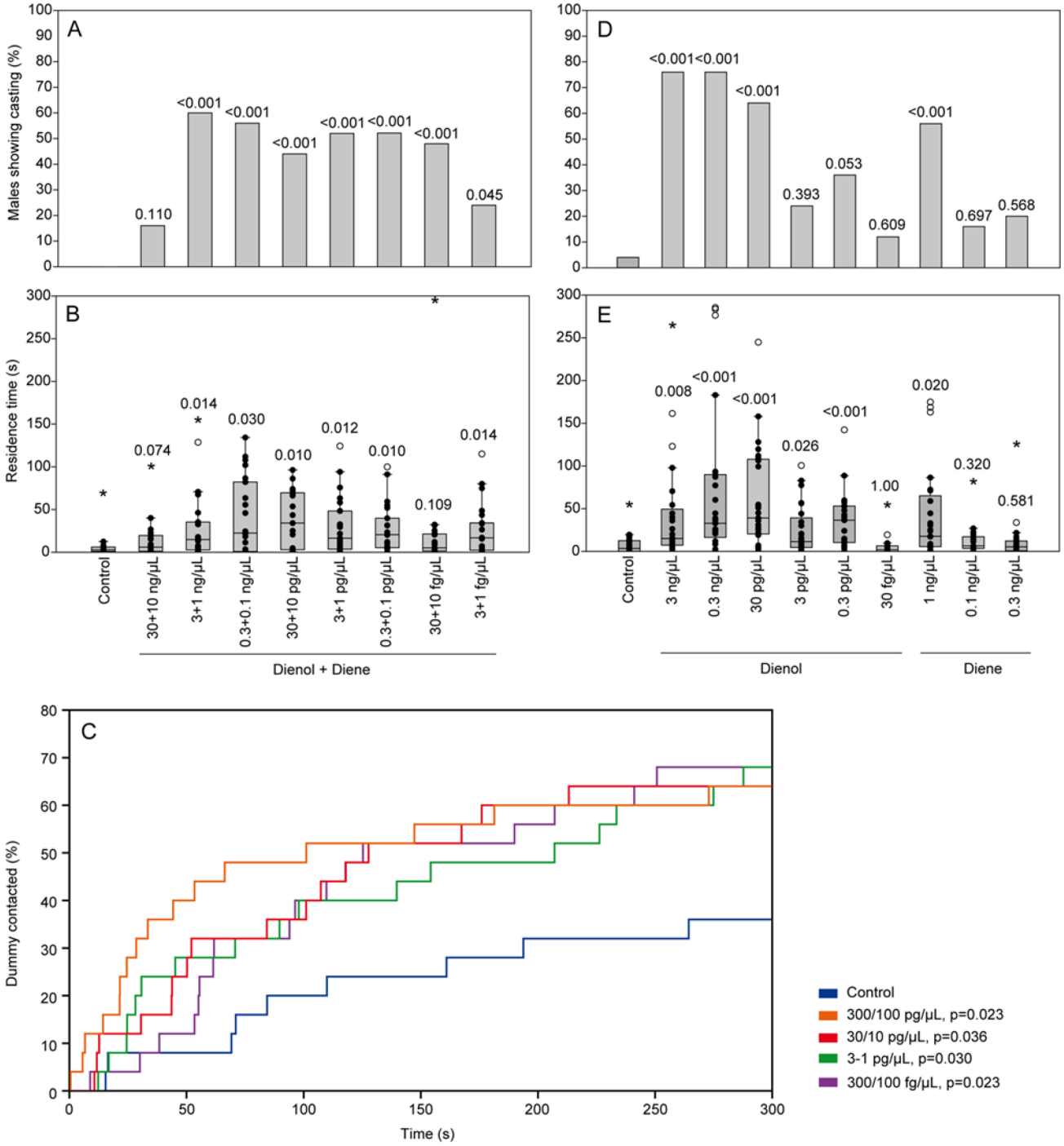


Fig. 3. Behavioral activity of synthesized pheromone compounds in bioassays. Panels A and D show the percentage of *T. turkestanica* males showing casting behavior towards dummies treated with the pure solvent (control) or different concentrations of (**A**) a 3:1 mixture of dienol **1** and diene **2** or (**D**) the two compounds alone. Comparisons between treatments and controls by multiple Fisher's exact tests after sequential Bonferroni correction (*p*-values above columns, $n = 25$). Panels (**B**) and (**E**) show summed residence times of males in the inner circle of the arena or on a dummy recorded in the same experiment. Box-and-whisker plots show 25 and 75% quartiles (upper and lower end of the boxes), median (horizontal line in between), 1.5× the interquartile range (whiskers), and outliers (° means > 1.5 × and * means > 3 × interquartile range). Comparisons between treatments and controls by Kruskal-Wallis H-test followed by multiple Mann-Whitney U-tests with sequential Bonferroni correction (*p*-values above boxes). Panel (**C**) shows the percentage of males that had contact with one of the dummies as a function of observation time. Comparison between treatments and control by Kaplan-Meier survival analysis and Log rank tests (concentrations of the 3:1 mixture of **1** and **2** as well as *p*-values given beneath the graph).

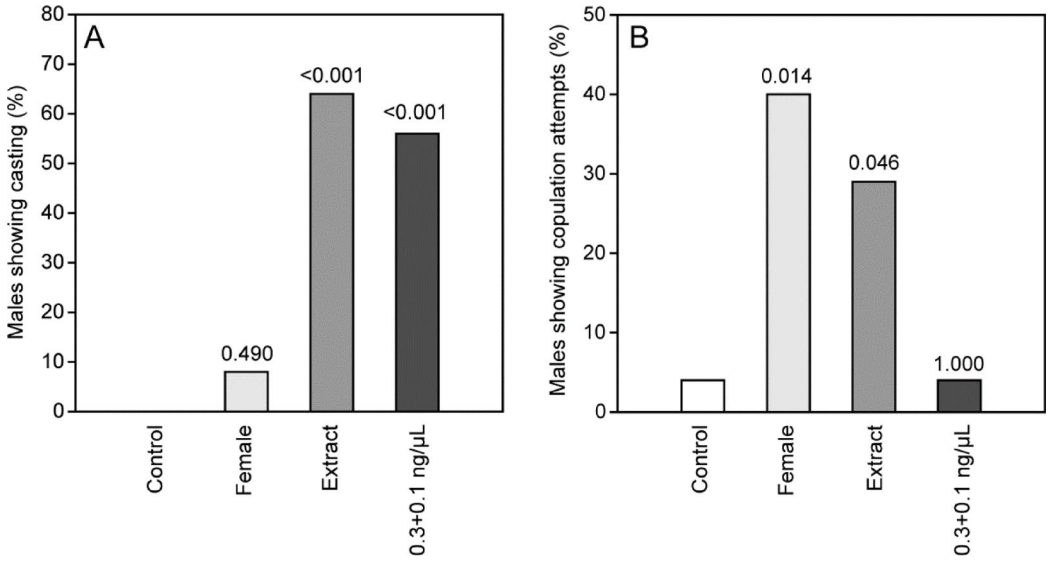


Fig. 4. Behavioral activity of dead unwashed females, female-derived extracts, and synthetic pheromone. Percentage of *T. turkestanica* males showing (**A**) casting behavior in response to and (**B**) copulation attempts with dead unwashed females and dummies treated with two equivalents of a female-derived dichloromethane extract or a 3:1 mixture of dienol **1** and diene **2** (0.3 + 0.1 ng/µL). Comparisons between treatments and control (solvent-treated dummies) by multiple Fisher's exact tests after sequential Bonferroni correction (*p*-values above columns, $n = 25$).

At the lowest bioactive concentration (3 + 1 fg/µL), the calculated maximum amount of pheromone on one of the 60 dummies treated per batch is 500 + 170 ag (1 attogram = 10^{-18} g). But in practice, the dose per dummy must have been considerably lower as much pheromone is left behind on the glass insert during coating of the dummies. This demonstrates the extreme sensitivity of *T. turkestanica* males for the female sex pheromone. Interestingly, unwashed dead virgin females did not elicit any casting in males while extracts from females did (Chi² contingency table test: $Chi^2 = 41.889$, df = 3, $n = 25$, $p < 0.0001$, Fig. 4A, Table S7 in the Supplementary Information) suggesting a glandular origin and an active release of the pheromone by the females.

While the 3:1 mixture of **1** and **2** attracted and arrested males and elicited the putative courtship element casting, male copulation attempts did not occur significantly more often on pheromone-treated dummies than on solvent treated controls. In contrast, males tried to copulate with unwashed dead females and with dummies treated with female-derived extract which also elicited casting behavior (Chi² contingency table test: $Chi^2 = 16.5$, df = 3, $n = 25$, $p = 0.00090$, Fig. 4B, Table S7 in the Supplementary Information). Hence, other, hitherto unknown compounds are involved in the initiation of copulation behavior in *T. turkestanica*. Candidates for this are cuticular hydrocarbons that have been demonstrated to elicit mating behavior in several species of parasitoid wasps, either alone[26–29], or in combination with more polar lipids such as triacyl glycerides[30].

By establishing the absolute configuration and demonstrating the behavioral activity of two previously reported natural products, the sex pheromone of a minute *Trichogramma* parasitoid wasp has now been fully elucidated. Remarkably, pheromone identification was accomplished although never more than 4 ng of a mixture of the two compounds could be isolated from the wasps. The chemical motif, a (mono-oxygenated) multiple-methyl substituted C12 or C13 alkane, occurs more frequently in sex pheromones of insects[19,31–34]. The presented synthesis contributes to a more efficient access to this class of compounds. A 3 + 1 mixture of **1** and **2** showed behavioral activity in males in triggering attraction, arrestment, and courtship down to low femtogram-levels. Observations of behavioral activity of sex pheromones at femtogram-levels are scarce if not absent[33]. A behavioral response at such low doses implies extremely high binding constants of **1** and **2** to their putative receptors. Insect olfactory neurons are extremely sensitive to pheromones. Calculations on the sensitivity of male silk moth (*Bombyx mori*) antennae to the female sex pheromone bombykol revealed that sensilla respond to single pheromone molecules[35] but for behavioral effects much higher amounts are required. The structure elucidation of the *T. turkestanica* pheromone will foster further chemo-ecological studies of *Trichogramma* species and may help to fill taxonomic gaps in this genus.

Methods

Insects for headspace sampling

Host eggs on paper cards, parasitized by *T. turkestanica* (Hymenoptera: Trichogrammatidae) were purchased at Nuetzlinge.de, (Sautter & Stepper GmbH, Ammerbuch, Germany). Species identity was confirmed by PCR (see section Insects for bioassays). The paper cards with host eggs were transferred into glass tubes (350608 * X500 PYREX 18 × 150 mm Disposable Rimless Culture Tub Fischer Scientific BV, the Netherlands) with cotton wool stoppers, provided with droplets of honey/water 50/50 and kept in an incubator at 22 ± 1 °C, 60% RH, 16:8 h light: dark. After emergence, they were provided with eggs of the moth *Ephestia kuehniella* (Koppert Nederland

B.V., Berkel en Rodenrijs, The Netherlands). Eggs were left for parasitization in the tube with wasps for two days and then transferred to a new tube. Two weeks after parasitization, parasitized eggs could be selected from their dark color and were individually placed into small glass tubes (X100 Tube boro 5.1 10 × 75 × 0.5 mm rimmed Fisherbrand, Fisher Scientific BV, The Netherlands) with a droplet of honey/water 50/50 and closed with cotton wool stoppers. After emergence, tubes with a female wasp were identified under a stereo microscope. Next, these tubes were cooled on ice, whereafter the cold-anaesthetized female wasps were transferred into a 1.8 ml glass autosampler vial. The vial was kept on a cold plate at 4 °C until between 15 and 100 female wasps were inside. Then headspace volatiles were collected at 25 °C for 24–48 h by SPME (PDMS 100 µm, Merck) for subsequent pheromone analysis.

Insects for bioassays

Wasps used for bioassays were also obtained from Nuetzlinge.de (Sautter & Stepper GmbH, Ammerbuch, Germany). Wasps were offered as *T. evanescens*, but sequencing of the ITS-2 region revealed them to be *T. turkestanica.* Identification was done using the method described previously[36] with the primers 5'-TGTGAA CTGCAGGACACATG-'3 (forward) and 5'-GTCTTGCCTGCTCTGAG-'3 (reverse). Wasps were delivered as parasitized host eggs glued on pieces of cardboard (approximately 3000 eggs per card). Wasps were kept at 22 °C and natural light conditions (about 16 h light/ 8 h dark). Cards that were temporarily not needed were stored in a climate cabinet at 18 °C (light/dark cycle 16:8 h) to decelerate emergence. Isolation of naïve, virgin wasps of defined age for bioassays was accomplished by a slightly different procedure: The egg cards were cut into 1 × 1 cm pieces that were stored singly in Petri dishes (4.5 cm diameter). All existing wasps in a given dish were carefully removed from the egg card pieces with a paint brush. Individual wasps found thereafter were immediately isolated in 1.5 ml Eppendorf tubes without having had contact with other wasps. These wasps were considered being naïve, virgin and newly emerged. All wasps were tested only once in the bioassays within the first day after emergence.

Preparation of extracts and dummies for the bioassays

For the preparation of odorless dummies for the bioassays, batches of approximately 100 freeze-killed (–75 °C) females were transferred to 1.5 mL glass vials equipped with 300 µL glass micro inserts (Klaus Trott Chromatographie-Zubehör, Kriftel, Germany) and extracted twice for 30 min with 2 µL of dichloromethane per wasp. Between extractions, the supernatant was carefully removed using a GC on-column syringe (10 µL; Hamilton Company, Reno, Nevada, USA). To remove remaining traces of the pheromone, the same amount of solvent was applied to the wasps a third time, but this time removed immediately. The extracted dummies were carefully dried at room temperature and stored at – 20 °C until use. Female-derived extracts were carefully concentrated under a gentle stream of nitrogen to a concentration of 60 female equivalents per 20 µL and used for the treatment of dummies.

Treatment of dummies

To apply the synthetic pheromone candidates or female-derived extracts, 60 odorless dummies were transferred to a 1.5 mL glass vial with micro insert and covered with 20 µL of dilutions of the synthetic pheromone candidates (Table S1 in the Supplementary Information) or female-derived extract, respectively. Dienol **1** and diene **2** were tested in combination and alone. The tested ratio of 3:1 for **1** and **2** was adapted to the ratio reported in the headspace of virgin females[14]. After covering the dummies with the pheromone solution or extract, the solvent was evaporated at room temperature under a gentle stream of nitrogen. Treated dry dummies were frozen at – 20 °C and thawed again right before the experiments.

Bioassays

All bioassays were carried out in a round test arena made of acrylic glass (diameter 1 cm, height 3 mm) covered with a cover slip. The arena was divided into an outer and inner zone by a circular marking (diameter 5 mm). The arena was uniformly illuminated by two arms of point-shaped cold light sources (KL 1500 Hal with flexible light guides; Schott, Mainz, Germany). Lateral incidence of light was excluded by an opaque grey plastic cylinder (diameter 10 cm, height 6 cm). Before each bioassay, two equally treated dummies (female-derived extract, synthetic pheromone, or pure solvent for control) were placed in the center of the arena. After 5–7 min, a naïve male (1-d old, n = 25 for each treatment) was placed into the arena using a hairbrush and the observation started. The position and behavior of the male was observed for 5 min through a stereo microscope at 160-fold magnification. The time spent by the male in the outer circle, in the inner zone and on a dummy was recorded using The Observer XT15 observational software (Noldus, Wageningen, The Netherlands). Furthermore, it was recorded when the first contact (antennation or direct mounting) with one of the dummies occurred. Finally, it was recorded whether and how often the male showed casting (see video S1 in the Supplementary Information) or copulation attempts. Casting describes conspicuous zigzagging movements shown by many insect species when perceiving pheromones and other wind-borne odors [25] and has also been described in T. turkestanica males when exposed to virgin females [13,14].

Chiral GC–MS

Chiral GC–MS analyses were carried out on an Agilent Technologies 7820 A GC coupled with an Agilent Technologies 5977E MSD. The GC was equipped with a Supelco β-DEX 120 (20% permethylated β-cyclodextrin (2,3,6-tri-O-methyl) in SPB-35 poly(35% phenyl/65% dimethylsiloxane)) capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness). Sample introduction either via SPME 1.0 min splitless (wasp sampling) or manual injection of 1 µL (reference solutions). Injector 250 °C. Temperature program: 105 °C (0 min hold) to 160 °C

(0 min hold) at 0.5 °C/min. Carrier gas He at 10.2 psi (0.95 mL/min), constant linear velocity mode. Retention time of **1** 100.08 min.

Chiral GC-FID analyses

Chiral GC-FID analyses were carried out on an Agilent Technologies 7820 A GC-FID system equipped with Supelco β-DEX 120 (20% permethylated β-cyclodextrin (2,3,6-tri-O-methyl) in SPB-35 poly(35% phenyl/65% dimethylsiloxane)) capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness). Sample introduction either via SPME 1.0 min splitless (wasp sampling) or manual injection of 1 µL (reference solutions). Injector 250 °C. Temperature program: 45 °C (1 min hold) to 105 °C (0 min hold) at 10 °C/min, then to 160 °C (0 min hold) at 0.5 °C/min. Carrier gas H_2 at 7.90 psi (1.34 mL/min), constant linear velocity mode. Detector 250 °C. Retention times of **2** and **1** 34.09 and 99.68 min respectively.

Enantioselective synthesis

Experimental details on the synthesis of **1** and **2** and their intermediate products are described in detail in the Supplementary Information (see Supplementary Methods and Supplementary Figures S2–S17).

Statistics and reproducibility

Statistical analyses were done using PAST scientific software (version 5.0.2)[37]. As most data were not normally distributed based on the Shapiro-Wilk test, they were analyzed using two-sided non-parametric tests. The sample size for the experiments ($n = 25$ for all experiments) was estimated using G-power 3.1.9.7 scientific software[38] considering an α-error of 0.05, a minimum statistical power of 0.8 and effect sizes (Cohen's $d \geq 0.8$) obtained in similar experiments in previous studies. The times spent on the dummies and inside the inner zone was added as a proxy for arrestment of males and analyzed by Kruskal-Wallis H-test. If this test revealed significant differences, pairwise comparisons with the control were carried out using Mann-Whitney U tests with sequential Bonferroni-correction[39]. The same procedure was applied to analyze the number of castings per 5 min observation time. The proportion of males showing casting or mating attempts was compared between treatments and control using a Chi^2 contingency table test and subsequent multiple Fisher's exact tests with sequential Bonferroni correction for individual comparisons between treatments and control. The time until first contact (antennation or direct mounting) was used as a proxy for attractiveness and compared between control and pheromone treatment with a Kaplan-Meier survival analysis and subsequent Log rank test.

Data availability

All data, including raw data underlying the figures, can be found in the Supplementary Information.

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Author contributions

T.A.v.B, J.J.J.A.v.L., W.F., A.J.M and J.R. designed research; T.A.v.B, J.P.K., A.D., H.M.S., M.H., K.C. and H.B. performed research; T.A.v.B, J.P.K., A.D., M.H., K.C., H.B., W.F., A.J.M and J.R. analyzed data; T.A.v.B., A.J.M., and J.R. wrote the paper with input from all other authors.

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Correspondence and requests for materials should be addressed to T.A.B., A.J.M. or J.R.