Limited worldwide variation of pheromone signal and response in the oriental fruit moth, *Grapholita molesta*

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Abstract. The response of *Grapholita molesta* males to sex pheromone blends containing varying ratios of the minor pheromone component E8-12:Ac (0.4%, 5.4%, 10.4%, 30.4%, and 100.4%) relative to the major pheromone component, Z8-12:Ac, was tested in eight world locations. In all populations the 5.4%E blend attracted significantly more males than any other blend. The 10.4%E blend was the second best attractant and captured significantly more males than the remaining blends, except in one population. The 0.4%E blend generally attracted more males than the 30.4%E blend and this one more than the 100.4%E blend. Captures in the 100%E blend were few and not different from the hexane control, except in one population. In a wind tunnel test there was considerable cross-attraction among populations and no indication of assortative mating. A wind tunnel test with synthetic pheromone blends showed, as in the field, no population differences in response to the different %E isomer tested. Except for a minor difference in the quantity of Z8-12:OH, the less important of the two minor components, females from different populations produced similar pheromone blends. We conclude that sex pheromone signal and response of *G. molesta* are very similar across widely separated world populations, suggesting that little geographical specialization has occurred during the recent geographical expansion of this species.

Introduction

The oriental fruit moth, *Grapholita molesta* Busk, is a tortricid fruit pest that occurs between 20° and 60° latitude in both hemispheres. The center of origin of *G. molesta* is thought to be in northwest China from where it has spread around the world (Rothschild and Vickers, 1991; Komai, 1999; Zheng et al., 2013). It was first reported in Australia around 1910 (Bailey, 1979), in Brasil in 1943 (Reis et al., 1988), in Chile in 1970 (Gonzalez, 1980), in Spain and New Zealand in 1976 (Rubio et al., 1990; Russell, 1987), and in South Africa in 1990 (Blomefield and Geertsema, 1990). It was first detected in the eastern United States in 1913, it reached California by 1942, and it is now found in all peach-growing areas of the United States and Canada (Rothschild and Vickers, 1991).

G. molesta females release a pheromone blend composed of Z8-12:Ac, E8-12:Ac and Z8-12:OH at a ratio of 100:6:10, respectively, and a synthetic blend with these compounds is a strong and species-specific male attractant (Baker and Cardé, 1979; Linn and Roelofs, 1983). Synthetic sex pheromones are used for mating disruption of *G. molesta* over 50.000 hectares of peach and apple around the world (Witzgall et al., 2010). Small variations in the ratio of Z8-12:Ac relative to E8-12:Ac strongly affect male *G. molesta* pheromone response (Baker and Cardé, 1979), so blend ratio could affect control by mating disruption (Cardé and Minks, 1995). Moth species that occur over widely separated areas, like *G. molesta*, sometimes show geographical variation in pheromone production and response (Löfstedt, 1990; Cardé and Haynes 2004). Therefore knowledge of population variation of pheromone signal and response has important implications in pest control. This knowledge, in addition, advances the understanding of signal evolution and the

65 speciation process (Smadja and Butlin, 2009). Variation of in pheromone gland composition
66 and response to different pheromone component blends has been studied in *G. molesta*
67 populations from Australia, France, Korea and the USA (Beroza et al., 1973; Roelofs and
68 Cardé, 1974; Rothschild and Minks, 1977; Baker and Cardé, 1979, Han et al., 2001, Yang et
69 al., 2002). These studies show that, overall, pheromone composition and preferred blends
70 are similar across populations. In the light of new studies showing genetic differences
71 among world populations of *G. molesta* (Kirk et al., 2013: Zheng et al., 2013), we speculate
72 that existing differences in pheromone signal and response may have gone undetected in
73 the previous pheromone studies.

74 The objective of our study is to determine if there is variation of sex pheromone
75 signal and response across a wider geographical area than previously sampled, including
76 populations not tested before, such as Italy, New Zealand, Spain, Turkey, and Chile. In
77 order to reduce experimental variation, a common pheromone stock solution was used at
78 all the world locations. Wind tunnel tests with live females and synthetic pheromone, and
79 pheromone identification tests were performed to further explore pheromone variability
80 among distant populations.

Materials and methods

Field trapping. Field tests were carried out in 2011 and 2012 at 8 world locations (Table 1). The pheromone components of *G. molesta*, (Z)-8-dodecenyl acetate (Z8-12:Ac), (E)-8-dodecenyl acetate (E8-12:Ac) and (Z)-8-dodecenol (Z8-12:OH) (Baker and Cardé, 1979) were purchased from Opennatur (Lleida, Spain, Purity ≥ 99%). Analysis of Z8-12:Ac by gas chromatography-flame ionization detection (GC-FID) revealed a 0.38% content of E8-12:Ac. Analysis of E8-12:Ac revealed a 0.24% content of Z8-12:Ac. E8-12:Ac was added in varying quantities to a stock solution containing 24 mg of Z8-12:Ac and 2.4 mg of Z8-12:OH in 15 ml of hexane (99% pure, Sigma-Aldrich, Spain) to obtain blends with 0.4%, 5.4%, 10.4%, 30.4%, and 100.4% E8-12:Ac relative to Z8-12:Ac. The same hexane used to make these dilutions served as a negative control. Pheromone blends were analyzed by GC-FID to confirm purity and correct blend composition. Hexane-rinsed red rubber septa (7 mm diameter Sigma-Aldrich, Madrid, Spain) were labeled with a different letter for each treatment using a permanent marker. Septa were loaded with 50 µl of solution so that they contained either hexane alone or blends with 80 µg of Z8-12:Ac + 8 µg of Z8-12:Ac + varying amounts (0 to 80 µg) of E8-12:Ac. The solvent was allowed to evaporate and septa were packed in glass bottles and stored at -20°C until shipped to their destinations. Septa for Chile and New Zealand were loaded on December 12, 2011 and septa for the other locations were loaded on August 6, 2011. Sampling took place between January 21 and March 28, 2012 in Chile and New Zealand, and between July 6 and October 1, 2011 in all other locations.

Delta traps were solid white except in the USA where they were orange. Trap color does not affect *G. molesta* response (Zhao et al., 2013). Traps were placed in apple,

nectarine or peach orchards in 3 to 5 plots per location. Traps within a plot were placed 20 to 40 m apart, and plots were at least 20 m apart from each other. Septa were placed directly on the sticky floor of the trap or where hung close to it, and were replaced by new ones between 1 and 2 times during the experiment. Traps were checked every 2 to 10 days and the sticky bottoms were replaced by new ones if there were captures. There was at least one complete rotation of the 6 treatments in all locations, except in the USA where the rotation was incomplete. Insects captured in the traps were counted and in some locations they were sexed and the species status identified according to genital morphology. Only male *G. molesta* were included in the analysis.

Insects for laboratory tests. Insects from France, Italy, Spain and the USA were reared in the laboratory for wind tunnel tests and to analyze pheromone composition (Table 1). The colonies from France and Spain were started new for this study from field collected insects, whereas insects from Italy and the USA were long-term laboratory colonies (Table 1). Larvae were reared in artificial diet (Ivaldi-Sender, 1974) and males and females were sexed at the pupal stage and kept in different environmental chambers inside well ventilated 2-litter clear plastic cages under a 16:8 L:D photoregime at 23 ± 1°C, with 10% sugar water solution for adult nutrition. Newly emerged adults were collected every 1-2 days and used when 2-5 days old.

Wind tunnel tests. The response of males to synthetic pheromone blends and to live females was tested in the wind tunnel. The wind tunnel consisted of a 150 x 45 x 45 cm (length x height x width) glass cage with a sliding door on one side. The floor was solid white, with no on purpose visual markings to aid insect flight. A 30-cm-diameter fan at the upwind end of the tunnel, and a 20-cm-diameter exhaust at the downwind end created a

0.35 m s^{-1} wind flow through the tunnel that was vented outside of the building after exiting the tunnel. The air entering the tunnel was unfiltered room air. Temperature inside of the tunnel (averaged for all the tests) was 21.63 ± 0.04°C (mean ± SEM). The wind tunnel was illuminated from above with fluorescent light bulbs producing 150 lux of white light inside. Tests were carried out during the last 4 hours of the photophase and occasionally into the first hour of the photophase, but in this case the daylight illumination was left on. Males were placed individually in 100-mm-long x 30-mm-wide cylindrical metal mosquito screen wire cages with one end permanently closed by rolling it on itself, and the other end covered with a removable aluminium lid. Males were placed in the cages 30 to 120 min before the test and taken to the wind tunnel room until tested.

To test the response of males to live females, a few minutes before the test 2- to 5-day-old unmated females were placed individually in glass tubes (20 mm diameter x 150 mm long) closed with a metal mosquito screen at one end and with an aluminium lid perforated with several 1.5-mm-diameter holes at the other end. Females were kept in a fume hood located outside the wind tunnel room and brought into the wind tunnel only for testing. Only females that were observed calling were used in the tests. The female tube was placed on and aluminium metal plate at the top of a 25-cm-tall metal wire platform (0.5-cm-mesh) and the tube was aligned with the wind flow so that its metal screen side faced downwind. For a given female 3 to 8 males were tested, one at a time, before discarding and replacing her with a new female. The order of the populations of females and males tested was randomized.

To test the response of males to synthetic blends containing 0.4%, 5.4%, 10.4%, 20.4%, 30.4%, 60.4%, or 100.4% E8-12:Ac, and 10% Z8-12:OH, relative to 100ng of Z8-

12:Ac, treatment solutions were applied in 10 µl loads to 10 x 15 mm hexane-rinsed filter papers (Whatman® No. 1). The filter paper was held by a 30 mm alligator clip and was let dry in a fume hood for 5-10 min before placing it in a 20ml odor-clean vial, where it remained until tested in the wind tunnel 5 to 180 minutes later. The base of the alligator clip was inserted in the slot of a 25 mm binder clip, itself fixed to a 70-mm-diameter aluminium metal plate located on top of a metal-wire platform similar to the one used for the females. Care was taken that the filter paper flat surface faced the wind flow to obtain a sufficiently turbulent odor plume. The action of taking the odor treatment out of the glass vial to fasten it to the binder clip was done inside the wind tunnel to minimize room air contamination. The response of 3 to 5 males to a filter paper treatment was tested before changing it for another treatment. At the end of a test day a filter paper had been exposed to between 6 and 12 males, so that filter papers were outside of the glass vial and exposed to the wind flow for a maximum of 24 to 36 minutes before being discarded. In a given day only one filter paper was used for each treatment, and new filter papers were loaded each day.

After placing the odor stimulus (live female or synthetic pheromone) in the upwind platform, the lid of the male cage was gently removed and the cage was placed in the wind tunnel with the open end facing upwind, on top of a metal wire platform similar to the one used for the odor source but 1.5 m downwind from it. The male was given 2 minutes to respond and then it was discarded. We recorded the number of males that landed on the filter paper or the tube containing the calling female.

171 At the end of the day the interior of the wind tunnel was cleaned with ethanol and
172 the exhaust fan was left on. All glass and metal utensils were thoroughly washed with
173 acetone and oven-dried at 200°C over-night.

174 *Pheromone gland composition*. Gland extracts of 2- to 5-day-old females were collected
175 during the last 3 h of the photophase. The tip of the abdomen containing the pheromone
176 gland tissue was excised carefully by pulling it from the abdomen with fine forceps.
177 Abdomen tips were placed in conical bottom vials either individually in 10-20 µL hexane or
178 in groups of 5 to 24 in 10-50 µL of hexane. Glands were extracted at room temperature for
179 30-120 min, and the vials with the extract were stored at -20°C until analyzed. For GC
180 analysis the sample was reduced to a few microliters under a gentle stream of N_2 and the
181 1-2 µl final concentrate was injected in a Hewlett Packard 5890 gas chromatograph
182 connected to a FID detector (Agilent Technologies, Madrid, Spain). The gas chromatograph
183 was equipped with a 30-m-long, 0.25 mm I.D., 0.25-µm film thickness DB-Wax column
184 (Agilent Technologies, Madrid, Spain). The constant Helium flow through the column was
185 1ml min^{-1}, and the injector and detector temperatures were 250 and 270°C respectively.
186 The oven temperature program increased from 50°C to 170°C at 20°C min^{-1}, and from
187 170°C to 230 °C at 10°C min^{-1}, and remained at this temperature for 10 min. Retention time
188 and quantification were estimated with the injection of synthetic standards and with the
189 internal standard, respectively. The retention times of E8-12:Ac, Z8-12:Ac and Z8-12:OH
190 were 9.08 min, 9.17.min and 9.78 min, respectively. For quantification of the pheromone
191 components the hexane used for extraction contained either 5 or 100 ng of pentadecane
192 (>99% pure, Sigma-Aldrich, Spain). Between 13 and 27 females were analyzed per
193 population, but the internal standard was used in only 8 extracts per population. Therefore

194 the sample size for quantification was N=8 and the sample size for ratio estimation was
195 N=13-27.

196

197 *Statistical analyses*. Field and wind tunnel data were analyzed with generalized linear
198 mixed models (GLMM) using the package lme4 in R (R development Core Team, 2008). For
199 the field data a Poisson distribution and logarithmic link function were used, and
200 pheromone blend, population and their interaction were considered as fixed effects while
201 date and plot nested to date were considered as random effects. For the wind tunnel tests
202 the logit link function was used to model binary responses (land/no land). The fixed part of
203 the model included pheromone blend, population, and their interaction for the test with
204 synthetic pheromone, or male and female population for the cross-attraction test with live
205 females. The random part in both wind tunnel models consisted of a random intercept per
206 day. Alternative models were rejected in terms of likelihood ratio tests, for nested models,
207 or Akaike Information Criterion (AIC), for non-nested models. Pearson coefficients and
208 overdiperssion further evaluated model fit. To perform contrasts between pairs of
209 treatments using the multcomp package of R, a single-step Bonferroni procedure for
210 correcting for multiple testing was considered, setting general type I error at alpha = 0.05.
211 Pheromone gland content was analyzed with Kruskal-Wallis tests followed by multiple
212 comparison tests using kruskal.test and kruskalmcp packages, respectively, in R (alpha =
213 0.05). Original data and the R codes are available as Supplementary material.

214

Results

Field tests. Species identity was confirmed by means of genital morphology in Korea and in Spain2. In Spain1 100% of the insects were *G. molesta*. In Korea 3.06% of the captures were *G. dimorpha* and 1.94% were other undetermined leafroller species. Adults were sexed in Chile, Italy, Spain2, and USA1, where females constituted 0.84%, 2.01%, 1.35%, and 0% of total captures respectively, with no conspicuous difference among treatments. The total number of males captured ranged between 208 in USA1 and 6,549 in Chile.

In all populations the 5.4%E blend captured more males than any other blend (Tables 2 and S1-3). The 10.4%E blend followed the 5.4%E blend in number of captures and it attracted more males than the 0.4%E, 30.4%E and 100.4%E blends in all the populations except in the Turkey where the 10%E blend did not differ from the 0.4%E blend. In Chile, Italy1, Korea, New Zealand, Spain2, and Turkey the 0.4%E blend attracted more males than the 30.4%E blend, whereas in Spain1 and USA1 these two blends attracted similar number of males. Hexane captured 0.26% of all the males. The 0.4%E blend always attracted more males than hexane, except in USA1, whereas the 100.4%E blend was better than hexane just in one population (Chile), and the 30.4%E blend was better than hexane in half of the populations. The 100%E, 30%E, and 0.4%E blends captured 1%, 2.8% and 10% of all the males. Better performance of the 5.4%E and 10.4%E blends relative to the other blends was consistent throughout the season except for discrete sampling dates when the 0.4%E and 30.4%E blend captured similar number of males as the 5.4%E and 10.4%E blends (Figure S1).

Wind tunnel response to live females. Percentages of cross-attraction ranged between 61% and 95% (Tables 3 and S4-6), and there was no obvious pattern of assortative mating among populations. In two populations (Italy2 and Spain2) females attracted less males from the USA3 than males from their own population, but this correlates with males from the USA3 having lower levels of response than males from the other populations, and they responded better to France females than to females from their own population. French males responded better to their own females than to USA3 females, but USA3 females were overall less attractive than females from the other populations.

Wind tunnel response to synthetic blends. The response of males to pheromone blends containing different ratios of the E isomer in the wind tunnel was very similar among the 4 populations tested (Tables 4, and S7-9). The 5.4%E, 10%E and 20.4%E blends were preferred, with few exceptions, over the other blends, and were followed in preference by the 30.4%E blend. The other blends (0.4%E, 10.4%E, and 100.4%E) were the least attractive. This is a similar patter as that observed in the field, except that in the wind tunnel males responded to a broader range of %E isomer than in the field.

Pheromone gland composition. Females from the four populations produced, in general, similar quantities and ratios of the 3 pheromone components (Table 5). The only difference was for the minor component Z8-12:OH which was less abundant in absolute and relative terms in USA3 females than in females from two of the other populations.

Discussion

Our study shows that in *G. molesta* there is limited population variation in pheromone composition and response across widely separated world areas. The population effect on the ratio of pheromone components in female gland extracts, although significant, was small and probably insufficient to influence male choice. In fact, cross-attraction among males and females of North American and European origin demonstrates that the small differences in blend composition between them do not prevent interpopulation attraction. Male response to artificial blends was remarkably similar among populations, both in the field and in the wind tunnel, further demonstrating the limited phenotypic variation present among geographically distant populations of *G. molesta*. Our study includes populations for which pheromone composition or response have already been studied (France, Korea and the USA), and populations that are studied for the first time (Chile, New Zealand, Spain, and Turkey), but populations from Australia, Brazil, Canada, China, Eastern Europe, Japan, and South Africa have not been examined yet. Therefore larger population differences in pheromone response and signal than what has been found for *G. molesta* may be possible.

Our results show that a 100:5:10 ratio of Z8-12:Ac, E8-12:Ac and Z8-12:OH, respectively, will be equally efficient at monitoring *G. molesta* in most regions of the world. Our study confirms previous observations made in Australia, Korea and the USA where the optimal proportion of the E isomer relative to the Z isomer is close to 5-6% and that lower or higher percentages of E8-12:Ac result in gradual decreases in response (Beroza et al., 1973; Roelofs and Cardé, 1974; Baker and Cardé, 1979; Yang et al., 2002). E8-12:Ac seems to be essential for male attraction because blends containing no, or undetectable levels of E8-12:Ac (as determined by chromatography) do not attract males

280 (Cardé et al., 1975a; Baker and Cardé, 1979; Linn and Roelofs, 1981). However in our study
281 a significant number of males were more attracted to the blend containing just 0.4% of the
282 E-isomer than to hexane, and other studies show that *G. molesta* can respond to blends
283 containing as low as 0.04%E (Baker et al., 1981). While it is clear that a minimum quantity
284 of E8-12:Ac is needed to make a minimally attractive blend with Z8-12:Ac (i.e., one that is
285 significantly more attractive than a negative control), the exact minimum ratio of E8-12:Ac
286 to Z8-12:Ac that produces a response in *G. molesta* males has yet to be determined.

287 The 30.4%E and 100.4%E blends captured significantly more than hexane, indicating
288 that some males are attracted by very high percentages of E-isomer in the blend. In our
289 wind tunnel test percentages of landing as large as 75% to the 30.4%E blend and 23% to
290 the 100.4%E blend (Italy2) were also observed. Response to such high ratios of E-isomer
291 contrasts with previous studies that often show almost complete lack of responses to 20%
292 or higher E-isomer in field and wind tunnel tests (Roelofs and Cardé, 1974; Baker and
293 Cardé, 1979; Linn and Roelofs, 1981; Baker et al., 1981). Sex pheromone response
294 polymorphism (with regard to percentage of E8-12:Ac) was disproved in *G. molesta* (Cardé
295 et al., 1976), but only a narrow range of E-isomer proportions were used in that study, and
296 all of them close to the optimal ratio (3, 8 and 11%). In view of our results it may be worth
297 repeating the experiment of Cardé et al. (1976) but with a wider range of E-isomer ratios to
298 retest pheromone response polymorphism in *G. molesta*. Rare males that respond to
299 extreme ratios of the E isomer but that also respond to the optimal blend ratio have been
300 described in *Ostrinia* spp. (Linn et al., 2007). Whether the response of *G. molesta* males to
301 extreme pheromone blend ratios in the wild is explained by the presence of rare males
302 needs to be determined.

303 Whereas a precise ratio of the E isomer is important for attraction, the role of Z8-
304 12:OH as a pheromone components is less clear. Support for the importance of this alcohol
305 in attraction comes from studies that show that calling females release it (Baker et al.,
306 1980), that males do not respond to a blend containing no Z8-12:OH, and that just a small
307 percentage of the alcohol (1-3%) is needed to increase male attraction significantly (Baker
308 and Cardé, 1979; Linn and Roelofs, 1983). On the other hand studies showing that Z8-
309 12:OH is not necessary for attraction (Roelofs and Cardé, 1974; Yang et al., 2002), that its
310 proportion in the blend can vary widely without affecting male response (Linn and Roelofs,
311 1983), and that females do not release it (Lacey and Sanders, 1992), question the status of
312 this alcohol as a pheromone component. A second alcohol, 12:OH, also released by females
313 (Baker et al., 1980) has been shown to play a role in precopulatory behavior when male *G.*
314 *molesta* is near the odour source (Cardé et al., 1975b). Baker and Cardé (1979) indicate
315 that the role of the two alcohols depends on the presence of each other and on the ratio of
316 E8-12:Ac to Z8-12:Ac. Z8-12:OH is a strong inhibitor for *G. prunivora* (Baker and Cardé,
317 1979), further challenging its role in *G. molesta* intraspecific communication. Despite the
318 questionable status of Z8-12:OH as a true pheromone component, it is clear from this and
319 other studies that a blend containing 10% of this compound is an affective attractant for *G.*
320 *molesta*.

321 The difference between wind tunnel and field tests in response to different
322 percentages of the E isomer could have different causes. Linn et al. (1988) showed that
323 wind tunnel temperature affects the response width of *G. molesta* to different percentages
324 of the E isomer, so that at 21°C almost no males respond to the 20%E blend (which was the
325 highest percentage of E that they used), whereas at 26°C about 50% of the males
326 responded to the 20%E blend. A similar result was observed in the field (Linn et al., 1988).

The temperature in our wind tunnel was close to 22°C which, according to Linn et al. (1988), should result in a relatively narrow response, however in our wind tunnel tests *G. molesta* responded to a broader ratio of the E isomer than in the field, so temperature on its own does not seem to explain the different breath of response between field and laboratory studies. Rubber septum dispensers were used in the field, whereas filter paper was used in the wind tunnel, but dispenser type is not either a likely cause for the differences between field and laboratory tests: Z8-12:Ac and E8-12:Ac have similar emission rates from a rubber septum (McDonough and Butler, 1983; Baker et al., 1980) they probably do so from a filter paper. Another factor that could explain differences between field and laboratory studies is that in the wind tunnel males were exposed to a single pheromone blend and always to a maximum distance of 1.5 m, whereas in the field males probably contact pheromone plumes from several females, and from several distances. Possibly the "forced" stimulus exposure in the wind tunnel could alter response specificity relative to the natural conditions. In *Ostrinia nubilalis* (Hübner) Z-strain males (those responding to a blend composed mainly of Z11-14:Ac) would rarely respond to the E-blend (composed mainly of E11-14:Ac), but if they are already flying to the Z-blend and are then suddenly swapped to the E-blend, they will complete their flight and contact the odor source as if they were flying to the Z-blend (Kárpáti et al., 2013), so it is possible that in *G. molesta*, and other moth species, unnatural exposure to a suboptimal blend ratio in the wind tunnel could alter their normal blend ratio response, and this could explain the difference between field and laboratory tests. A fourth possibility to explain the different breath of response of *G. molesta* to E-isomer ratios in the field and in the lab is that in the field there is a ubiquitous presence of background plant odors, whereas in the wind tunnel these are normally absent. It is well known that plant volatiles can alter the response to sex

pheromone, usually synergizing it (Landolt and Phillips, 1997), as we have found in *G. molesta* (Varela et al., 2011), so it may be worth exploring the role of plant volatiles on the response of *G. molesta* to different pheromone blend compositions.

The quantity and ratio of pheromone compounds in gland extracts in our study is similar to that reported in other studies of this species (reviewed by Han et al., 2001). Females from an Eastern Canadian population of *G. molesta* contain 1.5 ng/female of Z8-12:Ac in their glands, and 2.9% and 5.8% of E8-12:Ac and Z8-12:OH, respectively, relative to Z8-12:Ac (El-Sayed and Trimble, 2002). Females from a Korean population contain 0.8 ng of Z8-12:Ac per female gland and 6.8% and 19% of E8-12:Ac and Z8-12:OH, respectively (Yang et al., 2002). These values are not too different from the average minimum 1.47 ng (USA3) and maximum 2.07 ng (Italy2) quantities of Z8-12:Ac that we have found in the gland extracts. The ratio of E8-12:Ac to Z8-12:Ac reported by El-Sayed and Trimble (2002) (2.9%) is somewhat lower than the ratios reported by us (from 5.63% in the USA3 to 6.76% in Spain2) or by Yan et al. (2002), and the ratio of Z8-12:OH that we report in here (approx. 18%) is similar to the ratio reported in Korea (Yan et al., 2002) but 3 times larger than that reported in Eastern Canada (El-Sayed and Trimble., 2002), so these are indications that the Eastern Canadian population may be different from other populations. Females release between 0.15 and 3.2 (USA) and 8 (Australia) ng/hr of Z8-12:Ac (Cardé et al., 1979; Baker et al., 1980; Lacey and Sanders, 1992), with E-isomer ratios of 7% (Cardé et al., 1979) and 4% (Lacey and Sanders, 1992), and Z8-12:OH ratios of 30% (Cardé et al., 1979), and 22% (Lacey and Sanders, 1992). It should be noted that, as indicated by other authors, synthetic blends must contain a higher quantity of the alcohol due to its 3-times faster evaporation rates from rubber septa than the acetates (Baker et al., 1980).

374 The limited population variation in sex pheromone signal and response of *G.*
375 *molesta* shown in this and in previous studies could be explained by the recent
376 introduction of *G. molesta* in a large part of its geographic range, not giving it enough time
377 to develop geographic differences. By contrast, several moth species show significant
378 population differences over distant areas (Cardé and Haynes, 2004). Perhaps the best
379 studied example of geographical variation of moth pheromone communication is the
380 turnip moth, *Agrotis segetum* (Denis and Schiffermüller) for which there is substantial
381 population variability in the ratio of the 5 pheromone components, and males tend to
382 prefer those blends that are similar to the ones produced by females from their own
383 population (Löfstedt et al., 1986; Tóth et al.,1992). Recent studies are demonstrating that
384 intraspecific variability in pheromone communication is associated with host specialization,
385 as for the Z and E strains of *O. nubilalis* (Leppik and Frérot, 2012), and the corn and rice
386 strains of *Spodoptera frugiperda* (Unbehend et al., 2013). Indeed, two factors that could
387 favor race formation in *G. molesta* are the use of several host plants and the limited
388 dispersal capability of this species. *G. molesta* predominantly uses two widely cultivated
389 host plants, apple (*Malus* spp.) and peach (*Prunus* spp.) (Rothschild and Vickers, 1994).
390 Typically *G. molesta*, attacks peach tree shoots (the preferred hosts and larval feeding
391 tissue) early in the season and then moves to apple trees late in the season where it feeds
392 on shoots and fruits (Rothschild and Vickers, 1994). In late years reports of *G. molesta*
393 attacking apples are increasing (Myers et al., 2006a; Bellerose et al., 2007). Conceivably in
394 areas where only one of these two hosts occurs there is an opportunity for host-race
395 formation, further induced by the different phenology and suitability of each host for larval
396 development (Myers et al., 2006b). In *Cydia pomonella* (L.), which is closely related to *G.*
397 *molesta*, apple and walnut host races have been described (Phillips and Barnes, 1975), but

whether these races have divergent pheromone signals is not known. A second factor that could favor race formation in *G. molesta* is the limited movement of this pest. Dispersal of *G. molesta* is rather low, just a few hundred meters, and they tend to remain in the same field over the season (Ellis and Hull, 2012). The world-wide dispersal of *G. molesta* is thought to be caused by human introductions (Kirk et al., 2013), but once in an area the individuals do not move too much. Considering the limited self-dispersal of *G. molesta* and their main use of two different host plants it could be anticipated that populations from areas where only one of the two hosts is present may eventually become genetically and reproductively isolated with the possibility of evolving separate pheromone blends.

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Supplementary results index

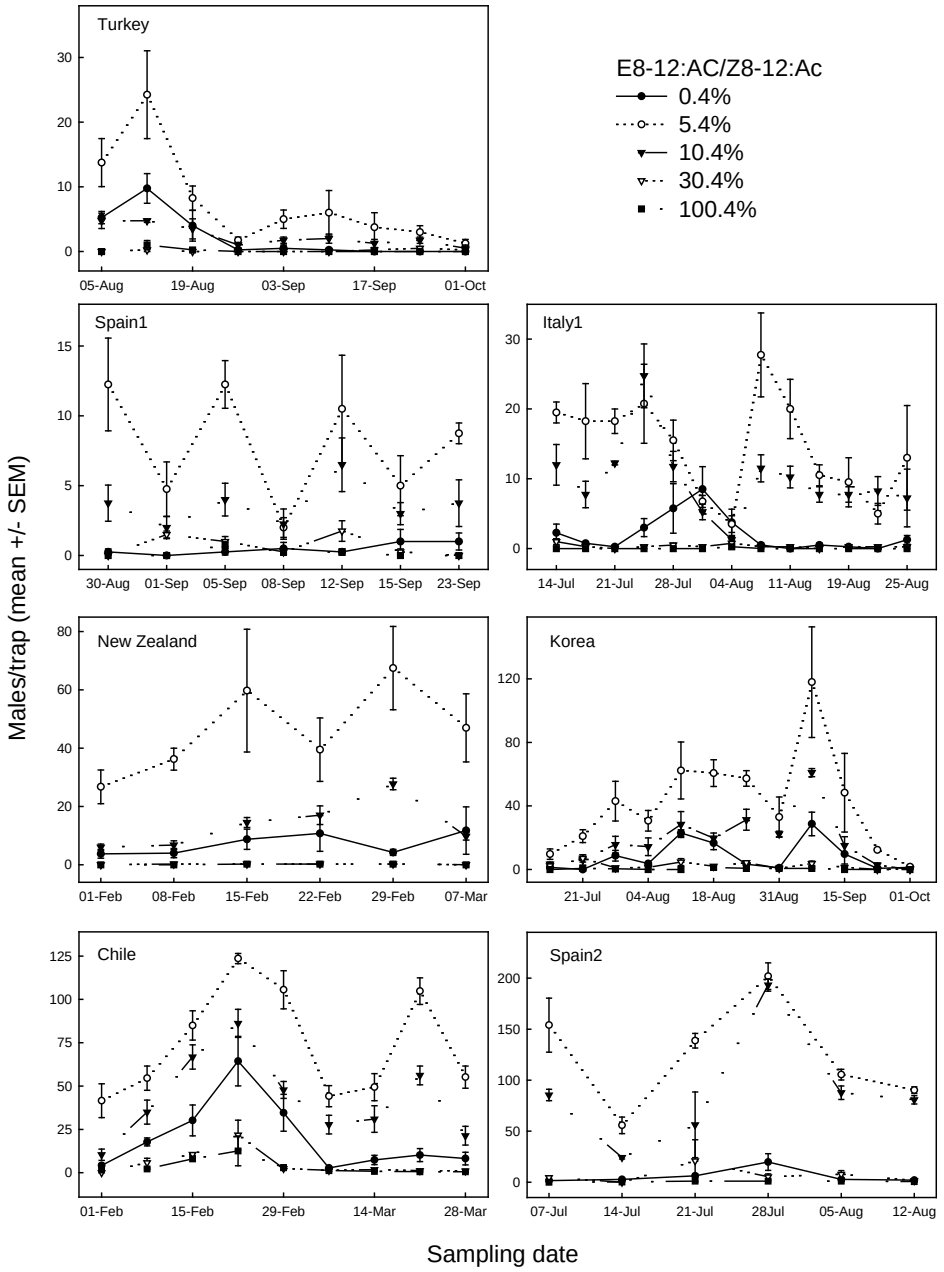

Figure S1. Captures of Grapholina molesta in pheromone traps at 7 world locations. Traps baited with different porcentages (0.4 to 100.4%) of E8-12:Ac respect to the major compound, Z8-12:Ac

	Pheromone blend					
Population	Hexane	0.4%E	5.4%E	10.4%E	30.4%E	100.4%E
Chile	0.76 (0.15)	19.98 (3.59)	73.78 (5.07)	42.51 (3.93)	5.11 (1.37)	3.40 (1.07)
Italy	0.06 (0.03)	2.19 (0.55)	14.48 (1.37)	9.83 (0.93)	0.33 (0.08)	0.02 (0.02)
Korea	0.00 (0.00)	8.17 (1.78)	41.50 (6.31)	18.31 (2.96)	2.39 (0.45)	0.36 (0.14)
New Zealand	0.04 (0.04)	7.21 (1.81)	46.12 (5.51)	13.62 (1.73)	0.17 (0.08)	0.12 (0.07)
Spain1	0.00 (0.00)	0.46 (0.19)	7.93 (1.17)	3.61 (0.58)	0.68 (0.19)	0.18 (0.08)
Spain2	0.00 (0.00)	5.83 (1.88)	124.37 (11.14)	87.96 (12.21)	6.67 (3.52)	0.62 (0.17)
Turkey	0.08 (0.05)	2.22 (0.65)	7.44 (1.50)	2.36 (0.35)	0.14 (0.06)	0.14 (0.09)
USA1	0.80 (0.44)	0.20 (0.14)	13.90 (4.41)	5.70 (1.32)	0.10 (0.11)	0.10 (0.11)

Table S1. Mean (SEM) number of *G. molesta* males captured per date/plot/trap in traps from 8 world locations loaded with hexane or with 5 pheromone blends varying in the proportion of E8-12:Ac with respect to the major compound Z8-12:Ac.

Generalized linear mixed model fit by the Laplace approximation
Formula: male ~ 0 + trt.pop + (1 | date/plot)
Data: fields
AIC BIC logLik deviance
4955 5221 -2427 4855
Random effects:
Groups Name Variance Std.Dev.
plot:date (Intercept) 0.099518 0.31546
date (Intercept) 0.407104 0.63805
Number of obs: 1526, groups: plot:date, 255; date, 64

Parameter	Estimate	Std. Error	z value	Pr(>|z|)	
trt.popchile-0	2.8355	0.2243	12.639	< 2e-16	***
trt.popchile-10	3.5906	0.223	16.099	< 2e-16	***
trt.popchile-100	1.0646	0.2361	4.509	6.52E-06	***
trt.popchile-30	1.4723	0.2314	6.361	2.00E-10	***
trt.popchile-5	4.1419	0.2225	18.613	< 2e-16	***
trt.popchile-hexane	-0.4394	0.2804	-1.567	0.117081	
trt.popgirona-0	-0.9048	0.3762	-2.405	0.016158	*
trt.popgirona-10	1.1454	0.2728	4.199	2.68E-05	***
trt.popgirona-100	-1.8603	0.5145	-3.616	0.0003	***
trt.popgirona-30	-0.5253	0.3423	-1.535	0.124901	
trt.popgirona-5	1.9329	0.2627	7.359	1.86E-13	***
trt.popgirona-hexane	-3.4697	1.0323	-3.361	0.000776	***
trt.popitaly-0	0.6736	0.2101	3.206	0.001345	**
trt.popitaly-10	2.1536	0.1912	11.266	< 2e-16	***

trt.popitaly-100	-4.0827	1.0173	-4.013	5.99E-05	***
trt.popitaly-30	-1.2495	0.3057	-4.088	4.35E-05	***
trt.popitaly-5	2.5413	0.1895	13.41	< 2e-16	***
trt.popitaly-hexane	-2.9841	0.6067	-4.919	8.70E-07	***
trt.popKorea-0	1.6919	0.2049	8.258	< 2e-16	***
trt.popKorea-10	2.499	0.2002	12.482	< 2e-16	***
trt.popKorea-100	-1.4268	0.3399	-4.198	2.69E-05	***
trt.popKorea-30	0.4626	0.224	2.065	0.038939	*
trt.popKorea-5	3.3175	0.1981	16.748	< 2e-16	***
trt.popKorea-hexane	-3.9917	1.0192	-3.917	8.98E-05	***
trt.popLleida-0	1.6431	0.2859	5.747	9.11E-09	***
trt.popLleida-10	4.3564	0.274	15.898	< 2e-16	***
trt.popLleida-100	-0.5905	0.3759	-1.571	0.116189	
trt.popLleida-30	1.7766	0.2844	6.248	4.16E-10	***
trt.popLleida-5	4.7028	0.2738	17.178	< 2e-16	***
trt.popLleida-hexane	-3.2985	1.0366	-3.182	0.001463	**
trt.popnz-0	1.879	0.2836	6.625	3.48E-11	***
trt.popnz-10	2.5157	0.2788	9.023	< 2e-16	***
trt.popnz-100	-2.1757	0.6388	-3.406	0.000659	***
trt.popnz-30	-1.888	0.5698	-3.313	0.000922	***
trt.popnz-5	3.7351	0.2749	13.587	< 2e-16	***
trt.popnz-hexane	-3.2743	1.0367	-3.158	0.001586	**
trt.poporegon-0	-1.8191	0.8459	-2.15	0.031516	*
trt.poporegon-10	1.5309	0.4825	3.173	0.001511	**
trt.poporegon-100	-2.5122	1.1026	-2.278	0.022701	*
trt.poporegon-30	-2.5122	1.1026	-2.278	0.022701	*
trt.poporegon-5	2.4223	0.4717	5.136	2.81E-07	***
trt.poporegon-hexane	-0.4328	0.5834	-0.742	0.458201	
trt.popturkey-0	0.3633	0.2535	1.433	0.151947	
trt.popturkey-10	0.4239	0.2521	1.681	0.092671	.
trt.popturkey-100	-2.4093	0.5021	-4.799	1.60E-06	***
trt.popturkey-30	-2.4093	0.5021	-4.799	1.60E-06	***
trt.popturkey-5	1.5722	0.2356	6.674	2.50E-11	***
trt.popturkey-hexane	-2.9202	0.621	-4.703	2.57E-06	***

Table S2. Estimations from the fitted model GLMM on the number of males captured in the pheromone traps. The data shown in the main text (Table 2) are obtained by back-transforming Estimate and Std. Error from this table (e.g., $e^{estimate}$).

Population	Contrast	Estimate	Std. Error	z value	Pr(>|z|)	
Chile	0 vs 10	-0.75514	0.04044	-18.675	<0.001	***
Chile	0 vs 100	1.77084	0.08745	20.249	<0.001	***

Chile	0 vs 30	1.36321	0.07389	18.448	<0.001	***
Chile	0 vs 5	-1.30644	0.0376	-34.748	<0.001	***
Chile	0 vs hexane	3.27491	0.17471	18.745	<0.001	***
Chile	10 vs 100	2.52598	0.08402	30.065	<0.001	***
Chile	10 vs 30	2.11836	0.06979	30.353	<0.001	***
Chile	10 vs 5	-0.55129	0.0287	-19.206	<0.001	***
Chile	10 vs hexane	4.03006	0.17302	23.293	<0.001	***
Chile	100 vs 30	-0.40763	0.10433	-3.907	<0.001	***
Chile	100 vs 5	-3.07727	0.08269	-37.216	<0.001	***
Chile	100 vs hexane	1.50407	0.1896	7.933	<0.001	***
Chile	30 vs 5	-2.66965	0.06818	-39.153	<0.001	***
Chile	30 vs hexane	1.9117	0.18374	10.404	<0.001	***
Chile	5 vs hexane	4.58135	0.17237	26.578	<0.001	***
Spain1	0 vs 10	-2.0502	0.2948	-6.954	<0.001	***
Spain1	0 vs 100	0.9555	0.5265	1.815	0.3954	
Spain1	0 vs 30	-0.3795	0.3601	-1.054	0.8734	
Spain1	0 vs 5	-2.8378	0.2855	-9.939	<0.001	***
Spain1	0 vs hexane	2.5649	1.0383	2.47	0.1034	
Spain1	10 vs 100	3.0057	0.4584	6.557	<0.001	***
Spain1	10 vs 30	1.6707	0.2502	6.677	<0.001	***
Spain1	10 vs 5	-0.7876	0.1201	-6.558	<0.001	***
Spain1	10 vs hexane	4.6151	1.0055	4.59	<0.001	***
Spain1	100 vs 30	-1.335	0.5029	-2.655	0.0646	.
Spain1	100 vs 5	-3.7933	0.4525	-8.383	<0.001	***
Spain1	100 vs hexane	1.6094	1.0961	1.468	0.6295	
Spain1	30 vs 5	-2.4582	0.2392	-10.278	<0.001	***
Spain1	30 vs hexane	2.9444	1.0266	2.868	0.0353	*
Spain1	5 vs hexane	5.4027	1.0028	5.387	<0.001	***
Italy	0 vs 10	-1.48004	0.10748	-13.771	<0.001	***
Italy	0 vs 100	4.75632	1.00495	4.733	<0.001	***
Italy	0 vs 30	1.92311	0.2616	7.351	<0.001	***
Italy	0 vs 5	-1.86774	0.10451	-17.871	<0.001	***
Italy	0 vs hexane	3.6577	0.58569	6.245	<0.001	***
Italy	10 vs 100	6.23635	1.00114	6.229	<0.001	***
Italy	10 vs 30	3.40315	0.24658	13.801	<0.001	***
Italy	10 vs 5	-0.3877	0.05732	-6.763	<0.001	***
Italy	10 vs hexane	5.13774	0.57914	8.871	<0.001	***
Italy	100 vs 30	-2.83321	1.02916	-2.753	0.044	*
Italy	100 vs 5	-6.62405	1.00083	-6.619	<0.001	***
Italy	100 vs hexane	-1.09861	1.15489	-0.951	0.9075	
Italy	30 vs 5	-3.79084	0.2453	-15.454	<0.001	***
Italy	30 vs hexane	1.7346	0.62633	2.769	0.0422	*
Italy	5 vs hexane	5.52544	0.57859	9.55	<0.001	***

Korea	0 vs 10	-0.80714	0.07014	-11.508	<0.001	***
Korea	0 vs 100	3.11865	0.28343	11.003	<0.001	***
Korea	0 vs 30	1.22924	0.1226	10.026	<0.001	***
Korea	0 vs 5	-1.62563	0.06381	-25.478	<0.001	***
Korea	0 vs hexane	5.68358	1.00176	5.674	<0.001	***
Korea	10 vs 100	3.92578	0.28009	14.016	<0.001	***
Korea	10 vs 30	2.03638	0.11466	17.76	<0.001	***
Korea	10 vs 5	-0.81849	0.04677	-17.502	<0.001	***
Korea	10 vs hexane	6.49072	1.00082	6.485	<0.001	***
Korea	100 vs 30	-1.8894	0.2976	-6.349	<0.001	***
Korea	100 vs 5	-4.74427	0.27857	-17.031	<0.001	***
Korea	100 vs hexane	2.56493	1.03781	2.471	0.095	.
Korea	30 vs 5	-2.85487	0.1109	-25.743	<0.001	***
Korea	30 vs hexane	4.45433	1.00586	4.428	<0.001	***
Korea	5 vs hexane	7.3092	1.00039	7.306	<0.001	***
Spain2	0 vs 10	-2.71328	0.08727	-31.089	<0.001	***
Spain2	0 vs 100	2.23359	0.27168	8.221	<0.001	***
Spain2	0 vs 30	-0.13353	0.11573	-1.154	0.8088	
Spain2	0 vs 5	-3.05971	0.08648	-35.383	<0.001	***
Spain2	0 vs hexane	4.94164	1.00357	4.924	<0.001	***
Spain2	10 vs 100	4.94687	0.25912	19.091	<0.001	***
Spain2	10 vs 30	2.57974	0.082	31.461	<0.001	***
Spain2	10 vs 5	-0.34644	0.02844	-12.182	<0.001	***
Spain2	10 vs hexane	7.65492	1.00024	7.653	<0.001	***
Spain2	100 vs 30	-2.36712	0.27003	-8.766	<0.001	***
Spain2	100 vs 5	-5.29331	0.25885	-20.449	<0.001	***
Spain2	100 vs hexane	2.70805	1.0328	2.622	0.0614	.
Spain2	30 vs 5	-2.92618	0.08115	-36.06	<0.001	***
Spain2	30 vs hexane	5.07517	1.00312	5.059	<0.001	***
Spain2	5 vs hexane	8.00136	1.00017	8	<0.001	***
New Zealand	0 vs 10	-0.63667	0.09402	-6.772	<1e-04	***
New Zealand	0 vs 100	4.05469	0.58236	6.963	<1e-04	***
New Zealand	0 vs 30	3.76701	0.50577	7.448	<1e-04	***
New Zealand	0 vs 5	-1.85612	0.08176	-22.703	<1e-04	***
New Zealand	0 vs hexane	5.1533	1.00292	5.138	<1e-04	***
New Zealand	10 vs 100	4.69136	0.58002	8.088	<1e-04	***
New Zealand	10 vs 30	4.40368	0.50307	8.754	<1e-04	***
New Zealand	10 vs 5	-1.21945	0.06294	-19.374	<1e-04	***
New Zealand	10 vs hexane	5.78997	1.00156	5.781	<1e-04	***
New Zealand	100 vs 30	-0.28768	0.76379	-0.377	0.999	
New Zealand	100 vs 5	-5.91082	0.57816	-10.224	<1e-04	***
New Zealand	100 vs hexane	1.0986	1.15474	0.951	0.908	
New Zealand	30 vs 5	-5.62314	0.50092	-11.226	<1e-04	***

New Zealand	30 vs hexane	1.38628	1.11808	1.24	0.763	
New Zealand	5 vs hexane	7.00942	1.00049	7.006	<1e-04	***
USA	0 vs 10	-3.35E+00	7.20E-01	-4.655	<0.001	***
USA	0 vs 100	6.93E-01	1.23E+00	0.566	0.991	
USA	0 vs 30	6.93E-01	1.23E+00	0.566	0.991	
USA	0 vs 5	-4.24E+00	7.12E-01	-5.954	<0.001	***
USA	0 vs hexane	-1.39E+00	7.91E-01	-1.753	0.433	
USA	10 vs 100	4.04E+00	1.01E+00	4.007	<0.001	***
USA	10 vs 30	4.04E+00	1.01E+00	4.007	<0.001	***
USA	10 vs 5	-8.91E-01	1.57E-01	-5.666	<0.001	***
USA	10 vs hexane	1.96E+00	3.78E-01	5.2	<0.001	***
USA	100 vs 30	-2.71E-10	1.42E+00	0	1	
USA	100 vs 5	-4.93E+00	1.00E+00	-4.916	<0.001	***
USA	100 vs hexane	-2.08E+00	1.06E+00	-1.96	0.308	
USA	30 vs 5	-4.93E+00	1.00E+00	-4.916	<0.001	***
USA	30 vs hexane	-2.08E+00	1.06E+00	-1.96	0.308	
USA	5 vs hexane	2.86E+00	3.64E-01	7.851	<0.001	***
Turkey	0 vs 10	-6.06E-02	1.56E-01	-0.389	0.998	
Turkey	0 vs 100	2.77E+00	4.61E-01	6.01	<1e-05	***
Turkey	0 vs 30	2.77E+00	4.61E-01	6.01	<1e-05	***
Turkey	0 vs 5	-1.21E+00	1.28E-01	-9.482	<1e-05	***
Turkey	0 vs hexane	3.28E+00	5.89E-01	5.579	<1e-05	***
Turkey	10 vs 100	2.83E+00	4.61E-01	6.152	<1e-05	***
Turkey	10 vs 30	2.83E+00	4.61E-01	6.152	<1e-05	***
Turkey	10 vs 5	-1.15E+00	1.25E-01	-9.218	<1e-05	***
Turkey	10 vs hexane	3.34E+00	5.88E-01	5.688	<1e-05	***
Turkey	100 vs 30	-2.71E-09	6.33E-01	0	1	
Turkey	100 vs 5	-3.98E+00	4.52E-01	-8.814	<1e-05	***
Turkey	100 vs hexane	5.11E-01	7.31E-01	0.699	0.977	
Turkey	30 vs 5	-3.98E+00	4.52E-01	-8.814	<1e-05	***
Turkey	30 vs hexane	5.11E-01	7.31E-01	0.699	0.977	
Turkey	5 vs hexane	4.49E+00	5.81E-01	7.732	<1e-05	***

Table S3. Contrasts among treatments within population, corrected by multiple testing with a single-step method, for male *G. molesta* captured in pheromone traps loaded with different percentages of the E8-12:Ac isomer or hexane. For each population all possible paired differences are statistically assessed. P-values shown in the table are corrected.

	Male			
Female	France	Italy2	Spain2	USA2
France	0.89 (74/83)	0.86 (37/43)	0.88 (38/43)	0.74 (31/42)
Italy2	0.94 (44/47)	0.88 (78/89)	0.91 (43/47)	0.61 (27/44)

Spain2	0.89 (41/46)	0.81 (38/47)	0.78 (68/87)	0.73 (33/45)
USA2	0.66 (29/44)	0.70 (31/44)	0.77 (34/44)	0.61 (27/44)

Table S4. Proportion of males responding to live females in the wind tunnel (number individuals landed/total individuals tested). Males and females from 4 populations were cross-tested.

Generalized linear mixed model fit by the Laplace approximation
Formula: land ~ 0 + male.female + (1 | nday)
AIC BIC logLik deviance
804 884.4 -385 770
Random effects:
Groups Name Variance Std.Dev.
nday (Intercept) 0.54258 0.7366
Number of obs: 839, groups: nday, 109

Parameter	Estimate	Std.Error	z values	Pr(>|z|)	
male.femalefrance-france	2.368	0.449	5.270	0.000	***
male.femalefrance-Italy	2.870	0.712	4.030	0.000	***
male.femalefrance-lleida	2.235	0.590	3.790	0.000	***
male.femalefrance-USA	0.764	0.449	1.700	0.089	.
male.femaleitaly-france	1.952	0.557	3.510	0.000	***
male.femaleitaly-Italy	2.158	0.411	5.250	0.000	***
male.femaleitaly-lleida	1.537	0.497	3.090	0.002	**
male.femaleitaly-USA	1.031	0.470	2.200	0.028	*
male.femalelleida-france	2.189	0.591	3.700	0.000	***
male.femalelleida-Italy	2.540	0.636	3.990	0.000	***
male.femalelleida-lleida	1.409	0.353	3.990	0.000	***
male.femalelleida-USA	1.380	0.488	2.830	0.005	**
male.femaleUSA-france	1.107	0.476	2.320	0.020	*
male.femaleUSA-Italy	0.467	0.442	1.060	0.291	
male.femaleUSA-lleida	1.122	0.462	2.430	0.015	*
male.femaleUSA-USA	0.563	0.440	1.280	0.201	

Table S5. Maximum likelihood estimates of probability of landing for *G. molesta* males in a wind tunnel experiment in response to females from their own or from different populations. Table 4 of the main text is obtained from the back-transformed Estimate and its Std. Error (e.g., $e^{estimate}$ /1+$e^{estimate}$).

Contrast between females from France and females from each of the other three populations in their attraction to males from France
Estimate Std. Error z value Pr(>|z|)
fr vs it == 0 -0.501 0.842 -0.60 0.895
fr vs llei == 0 0.134 0.741 0.18 0.996

fr vs usa == 0 1.604 0.635 2.53 0.032 *

Contrast between females from Italy2 and females from each of the other three populations in their attraction to males from Italy2
Estimate Std. Error z value Pr(>|z|)
fr vs it == 0 0.2056 0.6920 0.30 0.98
it vs llei == 0 1.1270 0.6242 1.81 0.18
it vs usa == 0 -0.0314 0.7202 -0.04 1.00

Contrast between females from Spain2 and females from each of the other three populations in their attraction to males from Spin2
Estimate Std. Error z value Pr(>|z|)
fr vs llei == 0 -0.7802 0.6886 -1.13 0.57
it vs llei == 0 -1.1306 0.7272 -1.55 0.31
llei vs usa == 0 0.0294 0.6024 0.05 1.00

Contrast between females from USA2 and females from each of the other three populations in their attraction to males from USA2
Estimate Std. Error z value Pr(>|z|)
fr vs usa == 0 -1.6703 0.6486 -2.58 0.028 *
it vs usa == 0 0.0961 0.6236 0.15 0.997
llei vs usa == 0 -0.5590 0.6380 -0.88 0.716

Contrast between males from France and males from each of the other three populations responding to females from France
Estimate Std. Error z value Pr(>|z|)
fr vs it == 0 0.416 0.715 0.58 0.90
fr vs llei == 0 0.179 0.742 0.24 0.99
fr vs usa == 0 1.261 0.655 1.93 0.14

Contrast between males from Italy2 and males from each of the other three populations responding to females from Italy2
Estimate Std. Error z value Pr(>|z|)
fr vs it == 0 -0.712 0.822 -0.87 0.747
it vs llei == 0 -0.382 0.757 -0.50 0.935
it vs usa == 0 1.691 0.604 2.80 0.015 *

Contrast between males from Spain2 and males from each of the other three populations responding to females from Spain2
Estimate Std. Error z value Pr(>|z|)
fr vs llei == 0 -0.826 0.687 -1.20 0.52
it vs llei == 0 -0.128 0.610 -0.21 0.99
llei vs usa == 0 2.531 0.581 4.35 <1e-04 ***

Contrast between males from USA2 and males from each of the other the populations responding to females from USA2
Estimate Std. Error z value Pr(>|z|)
fr vs usa == 0 -0.201 0.629 -0.32 0.98
it vs usa == 0 -0.468 0.644 -0.73 0.81
usa vs usa == 0 -0.816 0.657 -1.24 0.46

Table S6. Differences between treatments corrected by multiple testing with a single-step method, for the number of males from 4 populations responding females from the same 4 populations. For each male

population we compared their response to females of their own population with their response to females from the other populations. For each female population we compared the attraction of males from their own population with that of males from the other populations. P-values shown in the table are corrected.

	Pheromone blend						
Population	0.4%E	5.4%E	10.4%E	20.4%E	30.4%E	60.4%E	100%E
France	0.13 (7/52)	0.65 (33/51)	0.77 (40/52)	0.67 (35/52)	0.33 (17/52)	0.12 (6/49)	0.02 (1/48)
Italy	0.13 (12/90)	0.75 (67/89)	0.85 (74/87)	0.75 (72/96)	0.75 (66/88)	0.49 (42/86)	0.23 (18/77)
Spain2	0.11 (6/53)	0.92 (47/51)	0.83 (40/48)	0.84 (37/44)	0.44 (23/52)	0.14 (7/50)	0.10 (5/50)
USA1	0.14 (7/50)	0.62 (32/52)	0.72 (39/54)	0.74 (28/38)	0.35 (19/55)	0.04 (2/47)	0.00 (0/31)

Table S7. Proportion of male landings on odor stimulus sources in the wind tunnel (number individuals landed/total individuals tested). Pheromone blends differed in the ratio of the E8-12:Ac isomer. Males from 4 populations were tested.

Generalized linear mixed model fit by the Laplace approximation
Formula: land ~ 0 + trt.pop + (1 | nday)
AIC BIC logLik deviance
1653 1810 -797.4 1595
Random effects:
Groups Name Variance Std.Dev.
nday (Intercept) 0.0903 0.3005
Number of obs: 1644, groups: nday, 59

Parameter	Estimate	Std. Error	z value	Pr(>|z|)	
trt.popfrance-0	-1.9278	0.4185	-4.61	4.10E-06	***
trt.popfrance-10	1.1873	0.3411	3.48	0.0005	***
trt.popfrance-100	-3.8114	1.0326	-3.69	0.00022	***
trt.popfrance-20	0.7033	0.3073	2.29	0.0221	*
trt.popfrance-30	-0.7599	0.3077	-2.47	0.01352	*
trt.popfrance-5	0.5772	0.3049	1.89	0.05836	.
trt.popfrance-60	-2.0465	0.4483	-4.57	5.00E-06	***
trt.popitaly-0	-1.9187	0.3201	-5.99	2.00E-09	***
trt.popitaly-10	1.7564	0.3104	5.66	1.50E-08	***
trt.popitaly-100	-1.2107	0.2829	-4.28	1.90E-05	***
trt.popitaly-20	1.1357	0.2459	4.62	3.90E-06	***
trt.popitaly-30	1.1133	0.2563	4.34	1.40E-05	***
trt.popitaly-5	1.1299	0.2559	4.42	1.00E-05	***

trt.popitaly-60	-0.0638	0.2261	-0.28	0.77792	
trt.poplleida-0	-2.0797	0.4485	-4.64	3.50E-06	***
trt.poplleida-10	1.6349	0.4026	4.06	4.90E-05	***
trt.poplleida-100	-2.2292	0.4862	-4.59	4.50E-06	***
trt.poplleida-20	1.7079	0.4275	3.99	6.50E-05	***
trt.poplleida-30	-0.2228	0.2964	-0.75	0.45229	
trt.poplleida-5	2.5087	0.5364	4.68	2.90E-06	***
trt.poplleida-60	-1.8262	0.4225	-4.32	1.50E-05	***
trt.popUSA-0	-1.9882	0.4294	-4.63	3.70E-06	***
trt.popUSA-10	0.9296	0.3252	2.86	0.00425	**
trt.popUSA-100	-3.509	1.0441	-3.36	0.00078	***
trt.popUSA-20	1.1223	0.3851	2.91	0.00357	**
trt.popUSA-30	-0.7268	0.3101	-2.34	0.0191	*
trt.popUSA-5	0.4208	0.308	1.37	0.17187	
trt.popUSA-60	-3.3163	0.7432	-4.46	8.10E-06	***

Table S8. Maximum likelihood estimates of probability of landing for *G. molesta* males in a wind tunnel experiment in response to different percentages of E8-12:Ac in synthetic pheromone. Table 3 of the main text is obtained from the back-transformed Estimate and its Std. Error ($e^{estimate}/1+e^{estimate}$).

Population	Contrast	Estimate	Std. Error	z value	Pr (>|z|)
France	0 vs 10	-3.11	0.53	-5.90	<0.001 ***
France	0 vs 100	1.89	1.11	1.70	0.59
France	0 vs 20	-2.63	0.51	-5.19	<0.001 ***
France	0 vs 30	-1.17	0.51	-2.30	0.22
France	0 vs 6	-2.50	0.51	-4.96	<0.001 ***
France	0 vs 60	0.12	0.60	0.20	1.00
France	10 vs 100	5.00	1.08	4.62	<0.001 ***
France	10 vs 20	0.48	0.45	1.09	0.93
France	10 vs 30	1.95	0.45	4.37	<0.001 ***
France	10 vs 6	0.61	0.44	1.38	0.80
France	10 vs 60	3.23	0.55	5.86	<0.001 ***
France	100 vs 20	-4.52	1.07	-4.21	<0.001 ***
France	100 vs 30	-3.05	1.07	-2.85	0.060 .
France	100 vs 6	-4.39	1.07	-4.10	<0.001 ***
France	100 vs 60	-1.77	1.12	-1.58	0.68
France	20 vs 30	1.46	0.42	3.48	0.008 **
France	20 vs 6	0.13	0.42	0.30	1.00
France	20 vs 60	2.75	0.53	5.17	<0.001 ***
France	30 vs 6	-1.34	0.42	-3.20	0.021 *
France	30 vs 60	1.29	0.53	2.42	0.18
France	6 vs 60	2.62	0.53	4.95	<0.001 ***
Italy	0 vs 10	-3.67	0.44	-8.42	<0.001 ***
Italy	0 vs 100	-0.71	0.42	-1.69	0.62
Italy	0 vs 20	-3.05	0.39	-7.75	<0.001 ***
Italy	0 vs 30	-3.03	0.40	-7.58	<0.001 ***
Italy	0 vs 6	-3.05	0.40	-7.63	<0.001 ***

Italy	0 vs 60	-1.85	0.38	-4.87	<0.001 ***
Italy	10 vs 100	2.97	0.41	7.22	<0.001 ***
Italy	10 vs 20	0.62	0.39	1.61	0.67
Italy	10 vs 30	0.64	0.39	1.64	0.65
Italy	10 vs 6	0.63	0.39	1.60	0.68
Italy	10 vs 60	1.82	0.37	4.88	<0.001 ***
Italy	100 vs 20	-2.35	0.36	-6.43	<0.001 ***
Italy	100 vs 30	-2.32	0.37	-6.25	<0.001 ***
Italy	100 vs 6	-2.34	0.37	-6.30	<0.001 ***
Italy	100 vs 60	-1.15	0.35	-3.27	0.0184 *
Italy	20 vs 30	0.02	0.34	0.06	1.00
Italy	20 vs 6	0.01	0.34	0.02	1.00
Italy	20 vs 60	1.20	0.32	3.72	0.0037 **
Italy	30 vs 6	-0.02	0.35	-0.05	1.00
Italy	30 vs 60	1.18	0.33	3.57	0.0064 **
Italy	6 vs 60	1.19	0.33	3.62	0.0054 **
Spain2	0 vs 10	-3.71	0.59	-6.33	<0.001 ***
Spain2	0 vs 100	0.15	0.65	0.23	1.00
Spain2	0 vs 20	-3.79	0.60	-6.27	<0.001 ***
Spain2	0 vs 30	-1.86	0.52	-3.57	0.0061 **
Spain2	0 vs 6	-4.59	0.69	-6.69	<0.001 ***
Spain2	0 vs 60	-0.25	0.60	-0.42	1.00
Spain2	10 vs 100	3.86	0.62	6.27	<0.001 ***
Spain2	10 vs 20	-0.07	0.57	-0.13	1.00
Spain2	10 vs 30	1.86	0.48	3.87	0.0021 **
Spain2	10 vs 6	-0.87	0.66	-1.33	0.83
Spain2	10 vs 60	3.46	0.57	6.10	<0.001 ***
Spain2	100 vs 20	-3.94	0.63	-6.22	<0.001 ***
Spain2	100 vs 30	-2.01	0.55	-3.63	0.0051 **
Spain2	100 vs 6	-4.74	0.71	-6.66	<0.001 ***
Spain2	100 vs 60	-0.40	0.63	-0.64	1.00
Spain2	20 vs 30	1.93	0.50	3.85	0.0021 **
Spain2	20 vs 6	-0.80	0.67	-1.19	0.89
Spain2	20 vs 60	3.53	0.58	6.04	<0.001 ***
Spain2	30 vs 6	-2.73	0.60	-4.58	<0.001 ***
Spain2	30 vs 60	1.60	0.50	3.23	0.0209 *
Spain2	6 vs 60	4.33	0.67	6.49	<0.001 ***
USA	0 vs 10	-2.91	0.52	-5.63	<0.001 ***
USA	0 vs 100	14.58	686.91	0.02	1.00
USA	0 vs 20	-3.11	0.57	-5.49	<0.001 ***
USA	0 vs 30	-1.26	0.51	-2.50	0.13
USA	0 vs 6	-2.41	0.51	-4.75	<0.001 ***
USA	0 vs 60	1.33	0.84	1.58	0.64
USA	10 vs 100	17.50	686.91	0.03	1.00
USA	10 vs 20	-0.19	0.49	-0.39	1.00
USA	10 vs 30	1.65	0.42	3.92	0.0012 **
USA	10 vs 6	0.51	0.42	1.20	0.87
USA	10 vs 60	4.24	0.80	5.32	<0.001 ***
USA	100 vs 20	-17.69	686.91	-0.03	1.00
USA	100 vs 30	-15.84	686.91	-0.02	1.00

USA	100 vs 6	-16.99	686.91	-0.02	1.00
USA	100 vs 60	-13.25	686.91	-0.02	1.00
USA	20 vs 30	1.85	0.48	3.83	0.0018 **
USA	20 vs 6	0.70	0.48	1.46	0.72
USA	20 vs 60	4.43	0.83	5.34	<0.001 ***
USA	30 vs 6	-1.15	0.41	-2.81	0.0570 .
USA	30 vs 60	2.59	0.79	3.28	0.0131 *
USA	6 vs 60	3.73	0.79	4.73	<0.001 ***

Table S9. Differences among treatments within population, corrected by multiple testing with a single-step method, for the number of males from 4 populations responding the synthetic pheromone blends varying in the proportion of the E8-12:Ac isomer. For each population all possible paired differences are statistically assessed. P-values shown in the table are corrected.

	Pheromone blend					
Population	Hexane	0.4%E	5.4%E	10.4%E	30.4%E	100.4%E
Chile	0.64 (1.32) f	17.04 (1.25) c	62.92 (1.25) a	36.26 (1.25) b	4.36 (1.26) d	2.90 (1.27) e
Italy1	0.05 (1.83) e	1.96 (1.23) c	12.7 (1.21) a	8.62 (1.21) b	0.29 (1.36) d	0.02 (2.77) e
Korea	0.02 (2.77) e	5.43 (1.23) c	27.59 (1.22) a	12.17 (1.22) b	1.59 (1.25) d	0.24 (1.4) e
New Zealand	0.04 (2.82) d	6.55 (1.33) c	41.89 (1.32) a	12.38 (1.32) b	0.15 (1.77) d	0.11 (1.89) d
Spain1	0.03 (2.81) d	0.40 (1.46) c	6.91 (1.30) a	3.14 (1.31) b	0.59 (1.41) cd	0.16 (1.67) cd
Spain2	0.04 (2.82) d	5.17 (1.33) c	110.26 (1.31) a	77.98 (1.32) b	5.91 (1.33) c	0.55 (1.46) d
Turkey	0.05 (1.86)d	1.44 (1.29) c	4.82 (1.27) a	1.53 (1.29) bc	0.09 (1.65) d	0.09 (1.65) d
USA1	0.65 (1.79) c	0.16 (2.33) c	11.27 (1.60) a	4.62 (1.62) b	0.08 (3.01) c	0.08 (3.01) c

Table 2. Expected mean (SEM) number of *G. molesta* male captures in pheromone traps as predicted by the estimated parameters of a GLMM model. Pheromone traps were baited with hexane or with sex pheromone blends containing different percentages of the E8-12:Ac isomer relative to a constant load of 80 ng of Z8-12:Ac and 8 ng of Z8-12:OH on rubber septa, and were deployed in 8 world locations. Within each population different letters indicate significant differences among treatments (Tukeys test, $P < 0.05$). Actual captures and further statistical results are shown in tables S1-S3.

Female population	Male population			
	France	Italy2	Spain2	USA2
France	**0.91 (0.03)**	0.88 (0.03)	0.90 (0.04)	0.75 (0.07) b
Italy2	0.95 (0.05)	**0.90 (0.03)**	0.93 (0.06)	0.61 (0.07) a
Spain2	0.90 (0.04)	0.82 (0.04)	**0.80 (0.04)**	0.75 (0.06) a
USA2	0.68 (0.06) b	0.74 (0.07)	0.80 (0.06)	**0.64 (0.07)**

Table 3. Expected probabilites (SEM) of landing for *G. molesta* males from four different populations in a wind tunnel experiment in response to calling females from the four populations. Probabilities were obtained from the estimated parameters of a GLMM model. The diagonal line (in bold) represents within-population attraction. Within a female population (i.e., row) when males from another population responded different than males from their own (in bold), the former population was labeled with an “a”. Within a male population (i.e., column) when males responded different to females from another population than to their own (in bold), the former population was labelled a “b”. Actual landing numbers and further statistical results are shown in tables S4-S6.

	Pheromone blend						
Population	0.4%E	5.4%E	10.4%E	20.4%E	30.4%E	60.4%E	100.4%E
France	0.13 (0.05) bc	0.64 (0.07) a	0.77 (0.06) a	0.67 (0.07) a	0.32 (0.06) b	0.11 (0.05) bc	0.02 (0.02) c
Italy2	0.13 (0.04)c	0.76 (0.05) a	0.85 (0.04) a	0.76 (0.04) a	0.75 (0.05) a	0.48 (0.05) b	0.23 (0.05) c
Spain2	0.11 (0.04) c	0.92 (0.04) a	0.84 (0.05) a	0.85 (0.05) a	0.44 (0.07) b	0.14 (0.05) c	0.09 (0.04) c
USA1	0.12 (0.05) cd	0.60 (0.07) ab	0.72 (0.06) a	0.75 (0.07) a	0.33 (0.06) bc	0.04 (0.03) d	0.03 (0.03) d

Table 4. Expected probabilities (SEM) of landing for *G. molesta* males in a wind tunnel experiment in response to different percentages of the E8-12:Ac isomer in synthetic pheromone. Probabilities were obtained from the estimated parameters of a GLMM model. Different letters in the same row indicate differences among treatments within populations (Tukeys test, $P < 0.05$). Actual landing numbers and further statistical results are shown in tables S6-S9.

Population	ng/female (% to major compound) (mean ± SEM)				
	Z8-12:Ac	E8-12:Ac		Z8-12:OH	
France	1.54 ± 0.18	0.10 ± 0.01	(6.22 ± 0.49)	0.33 ± 0.03 ab	(21.21 ±1.74) a
Italy2	2.07 ± 0.35	0.14 ± 0.02	(6.46 ± 0.25)	0.41 ± 0.04 a	(19.82 ± 1.27) a
Spain2	1.95 ± 0.35	0.13 ± 0.02	(6.76 ± 0.33)	0.36 ± 0.03 ab	(18.07 ± 3.09) ab
USA3	1.47 ± 0.49	0.08 ± 0.03	(5.63 ± 0.27)	0.21 ± 0.06 b	(15.30 ± 0.93) b
K.W. p-value	0.353	0.112	0.039*	0.015	0.002

Table 5. Quantity and percentage of pheromone components in gland extracts of *G. molesta* females from several populations. Different letters in a column indicate significant differences among populations ($P < 0.05$). *In this case the overall Kruskal-Wallis test was significant but there were no differences among treatment pairs.