ORIGINAL RESEARCH

Variation in Attraction to Host Plant Odors in an Invasive Moth Has a Genetic Basis and is Genetically Negatively Correlated with Fecundity

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Abstract Lepidopteran insects are major pests of agricultural crops, and mated female moths exploit plant volatiles to locate suitable hosts for oviposition. We investigated the heritability of odor-guided host location behavior and fecundity in the cosmopolitan oriental fruit moth *Grapholita* (*Cydia*) *molesta*, an oligophagous herbivore that attacks fruit trees. We used a full-sib/half-sib approach to estimate the heritability and the genetic correlation between these two traits. Results document a considerable genetic basis for olfactory attraction of females ($h^2 = 0.37 \pm 0.17$) and their fecundity ($h^2 = 0.32 \pm 0.13$), as well as a genetic trade-off between female attraction and fecundity ($r_g = -0.85 \pm 0.21$). These estimations were empirically corroborated by comparing two strains maintained in the laboratory for different numbers of generations. The long-term reared strain lost its olfactory discrimination ability but achieved significantly higher fecundity compared with the short-term reared strain. Our results highlight that genetic studies are relevant for understanding the evolution of odor-guided behavior in herbivore insects and for judging the promise of pest management strategies involving behavioral manipulation with plant volatiles.

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Introduction

Plants release volatile organic compounds that play multiple roles in interactions with associated animals. Insect herbivores exploit these volatiles to locate their host plants from a distance for feeding, mating and/or egg-laying (Bernays and Chapman 1997; Bruce et al. 2005). In recent years considerable progress has been made in elucidating the molecular mechanisms that underlie odor recognition and odor-guided behavior in herbivore insects (e.g. Anholt et al. 2003; Vosshall and Stocker 2007; Lavagnino et al. 2012). However, we still know relatively little about the genetic basis of this behavior and its contribution to the evolution of plant–insect herbivore interactions (Dambroski et al. 2005), particularly in lepidopteran species that are major pests in numerous crops worldwide.

In order to generate testable hypotheses about the evolution of innate behaviors associated with odor-guided host plant location by insect herbivores, we must first determine to what degree these behaviors are heritable, as it is genetic variance that provides the basis for any response to selection (Falconer and Mackay 1996). Heritability, the additive portion of polygenic variation, dictates the rate at which genetic change will occur in a given trait in a specific population, when selection for that trait is practiced (Falconer and Mackay 1996; Roff and Fairbairn 2007). Because it predicts the expected

phenotypic mean of the next generation from the phenotypes of the parents, it is the key genetic parameter that permits prediction of response to selection for that trait. Additionally, measurements of genetic correlations among traits allow understanding the evolution of a given suit of traits, as they reflect the degree of pleiotropy and linkage of genes underlying variation for the traits and are used in predicting the responses of those traits to selection (Falconer and Mackay 1996; Duckworth and Kruuk 2009; Colautti et al. 2010).

Lepidopteran species are key herbivores in many dicotyledonous crops including fruit trees (e.g. the codling moth *Cydia pomonella* L.), maize (e.g. the European corn borer *Ostrinia nubilalis* Hübner) and cotton (e.g. the bollworms *Helicoverpa* spp. and the cutworms and armyworms *Spodoptera* spp.). The oriental fruit moth *Cydia* (=*Grapholita*) *molesta* (Busck) (Lepidoptera: Tortricidae) is among the most damaging invasive fruit moth species (Rothschild and Vickers 1991). It has followed its hosts around the world and is currently expanding its distribution range, concomitantly with recent climate warming (Torriani et al. 2010). Thus, the economic relevance of this pest is expected to continue to increase in the next decades. The host range of the oriental fruit moth is confined to plant species of the family Rosaceae, mostly from the genera *Prunus* and *Pyrus*, and to one shrub of the family Myrtaceae (Rothschild and Vickers 1991; Natale et al. 2003). Because nearly all of its host plants belong to the family Rosaceae, the oriental fruit moth is an oligophagous pest species (Rothschild and Vickers 1991). The oriental fruit moth has recently been used as a model insect herbivore for studies on dispersal (Hughes and Dorn 2002; Torriani et al. 2010), thermal tolerance (Notter-Hausmann and Dorn 2010) and behavioral and neurophysiological responses to plant-derived odors (Piñero et al. 2008; Najar-Rodriguez et al. 2010, 2011).

Attraction of mated oriental fruit females to the primary host peach (*Prunus persica* L.) is guided mainly by olfactory cues, and the particular volatiles mediating this behavior have been identified (Natale et al. 2003; Piñero and Dorn 2007). Many aspects of the olfactory behavior of the female moths, however, are still unknown. Substantial differences in olfactory responses across oriental fruit moth individual females of similar age, mating and feeding status were previously observed both at the behavioral and neurophysiological level (Najar-Rodriguez et al. 2010, 2011). The origin of the inter-individual variation in olfactory responses was postulated to be adaptive and possibly genetically based. It could help female moths deal with qualitative and quantitative fluctuations in plant odor signals, and prevent plants evading herbivore attack.

A number of attractant-based approaches for insect herbivore pest management rely on the assumption that adult insect response to distinct volatiles is a species-specific trait, neglecting the option for potential significant intra-specific variations. Thus, knowledge of such potential genetically-based variation in olfactory attraction may determine the promise and the long-term success of tactics based on behavioral manipulation with plant volatiles such as odor traps for pest monitoring or mass-trapping based control.

In this study, we worked with the oriental fruit moth as a model organism to investigate whether and to what extent attraction of mated females to host plant odors has a genetic component. To address this question, we used a full-sib/half-sib approach to estimate heritability of female attraction to host plant odors in behavioral bioassays. Furthermore, we calculated the heritability of a key fitness-related life-history trait, the females' fecundity, and the genetic, residual and phenotypic correlations between this trait and female attraction to host plant odors. Finally, we corroborated results from the heritability studies and genetic correlation estimations empirically by comparing female odor attraction and fecundity between two strains of common origin, which were maintained for two different numbers of generations under the same conditions in the laboratory.

Materials and methods

Insects

The mated oriental fruit moth females used to measure the heritable component of female olfactory attraction and fecundity were obtained from a laboratory colony that had been reared at the ETH applied entomology for no more than 10 generations. The colony originated from individuals collected in peach orchards in Northern Italy (Emilia-Romagna region) in May 2009. Adult moths were kept in an insectary at 24 °C, 60 % RH and L:D 16:8 h. Water was provided ad libitum. Neonate larvae were grown on an agar-based artificial diet (Huber et al. 1972) that was devoid of any host plant cues and were kept under the same conditions as the adults.

Plant material

To obtain natural host plant volatiles, peach twigs [length (cm) 17.1 ± 0.5, weight (g) 5.42 ± 0.20, leaves number 8.81 ± 0.29] were cut from 2 year-old trees from the cultivar 'Red Heaven'. Trees were grown in downtown Zurich on the ETH campus. Trees were fertilized every 4 weeks (Blaukorn slow release fertilizer, N/P/K 12:12:17; 1.2 Mg; 6 Magnesium sulphate; 0.02 Boron; Omya AG, Safenwil, Switzerland). No plant protectants were applied, and only healthy plants were used. Twigs devoid of any damage were cut 10–15 min before the start of the bioassays and the cut ends of the twigs were wrapped with parafilm to minimize the emission of wound-induced volatiles.

Behavioral bioassay

The olfactory attraction of female oriental fruit moths to host plant odors was tested in a dual choice Y-tube olfactometer bioassay. The olfactometer consisted of a Y-shaped glass tube (diameter 2.5 cm, arm length 23 cm and stem length 23 cm), which was connected to two tubular glass tubes (diameter 6 cm and length 38 cm). These tubes were, in turn, connected to a glass chamber (diameter 10 cm and length 14 cm), in which the odor sources were placed. Charcoal-filtered, moistened air was drawn into each of the two glass chambers and Y-tube arms at a rate of 740 ± 10 mL/min at the entrance. Airflow rates were calibrated prior to and during experiments with an electronic flow meter (Agilent flow meter ADM 1000; Agilent Technologies, Centerville, DE, USA).

Bioassays were conducted during the period in the diel cycle when females are most active, i.e. at the transition of day to night conditions (Natale et al. 2003). Females were chosen at random from their rearing containers during the 2.5 h preceding scotophase, i.e. night conditions, and were brought into a dark bioassay room at 24–25 °C and 60–70 % RH (Piñero and Dorn 2009). Each female was allowed to acclimatise in the bioassay room for 10 min prior to the start of the experiment. The female was then released into the common arm of the Y-tube and was given the choice between clean air (blank) and odors from a plant sample. A 60-W red light bulb was placed above the olfactometer to observe the female for up to 10 min. A female was considered to have made a choice during this period if it entered either arm and crossed a score line drawn 3 cm from the intersection of the tube. By contrast, a female was considered to have not made a choice if it remained in the common arm of the Y-tube throughout the 10 min observation period (Bertschy et al. 1997). Naïve females that had never been exposed to any host plant odor prior to the bioassays were used for the experiments. A new plant sample was used for each individual female tested. The positions of the glass tubes as well as the position of the two arms of the olfactometer were systematically changed after testing 3–4 moths in order to prevent positional bias. After each day, all parts of the olfactometer that had come in contact with the moths were washed in a detergent solution, rinsed with acetone and hexane, and finally oven-dried for at least 12 h at 150 °C.

Heritable component of female olfactory attraction and fecundity

To test whether and to what degree the differences across oriental fruit moth female individuals on olfactory attraction and fecundity have a genetic basis, we estimated heritabilities of these two traits using a full-sib/half-sib design. Sibling designs provide a way to examine the genetic variance and covariance structure within a population, and they provide a baseline for further studies of complex life history adaptations (Dingle 1984).

The balanced design of full-sib/half-sib families used to calculate the heritabilities of female olfactory preference and fecundity was as follows. A half-sib family consisted of the offspring from two females mated to the same male, whereas a full-sib family consisted of the offspring from only one of the two females mated to the same male. First, 10 virgin males were each paired with two virgin females, with the males and females being randomly selected from the stock colony. Each group of three adults (one male plus two females) was placed in a separate plastic box (6 cm × 4 cm × 2 cm) that contained a moist cotton ball to prevent dehydration and was assigned a family number from 1 to 10. All plastic boxes were then transferred into a controlled climate chamber (Conviron Ltd., Winnipeg, MB, Canada) with L:D 16:8, 60 % RH, and 24 °C, for 3 days. Each male was mated with the two females during the 2.5 h preceding scotophase, i.e. the period when mating usually takes place. When mating lasted longer than 15 min, it was considered successfully completed. The mated females were then transferred into a separate box each, labelled as A or B, respectively, where each female was allowed to continue laying eggs until death. Water was provided ad libitum. It was not necessary to add host plant cues as oviposition stimulants, as preliminary trials indicated that the lack of such cues does not affect female oviposition behaviour. The fertility of all eggs that had been laid by each female was also checked to assure that each female had successfully mated.

Newly emerged larvae from each full-sib family (A or B) were kept separately in a plastic Petri dish (135 mm diameter, 18 mm height) that contained artificial diet to provide food for larval development. Corrugated cardboard strips were attached to the lid of the Petri dishes 1 week later in order to provide larvae with a place to pupate. Cardboard strips containing pupae were then removed, and all pupae were sexed, weighed and kept together in a plastic container (10 cm × 10 cm × 8 cm) until adult emergence.

Twenty newly emerged virgin females from each full-sib family were then randomly selected, weighed and transferred into separate plastic boxes (6 cm × 4 cm × 2 cm), where each female was paired with a randomly chosen, newly emerged virgin male from the stock population. 3–4 days later, when the females were between 4 and 5 days old (i.e. highly responsive to host plant odors), Y-tube olfactometer bioassays were conducted as previously described.

A total of 400 females, 20 females per full-sib family, were tested. Each day, however, only four females per family were tested as to minimize the effect of potential inter-day variation on family olfactory responses.

Furthermore, the same plant twig was offered to one female from each family at a given day, in order to minimize effects of potential inter-twig variation. Each plant twig was used only for one single day. Each female was tested only once and at the end of each day, all females that were tested were placed back into their respective boxes where they continued to lay eggs until their death. The total number of eggs laid per female (i.e. female fecundity) was then recorded.

Comparison of female olfactory attraction and fecundity between two strains differing in the number of generations maintained in the laboratory

To verify results from the heritability studies and genetic correlation estimations, we tested two strains of the oriental fruit moth that were maintained short-term and long-term, respectively, under the same conditions in the laboratory. Whereas the strain reared short-term was kept for less than 10 generations in the laboratory, the long-term strain was continuously reared for over 70 generations. These strains were collected in 2009 and 2007, respectively, in the same region of Northern Italy.

We used plant material from both the primary host (peach) and a secondary host (pear) as follows: fruit-bearing peach twigs [length (cm) 20.4 ± 0.9, weight (g) 83.24 ± 6.27, leaves number 19.50 ± 1.49, fruit diameter (cm) 4.4 ± 0.1, cultivar 'Red Heaven'] and fruit-bearing pear twigs [length (cm) 18.7 ± 0.7, weight (g) 117.87 ± 7.74, leaves number 20.2 ± 2.02, fruit diameter (cm) 4.9 ± 0.2, cultivar 'Harrow Sweet'], collected on-site in Southern Switzerland (Canton Ticino). Copper was applied to plants once in the spring for protection against diseases. The twigs were cut and wrapped with parafilm, placed inside plastic bags that contained wet cotton in order to reduce dehydration, and immediately brought to the laboratory for bioassays.

The olfactory attraction of the two strains was tested in Y-tube olfactometer as described above. A single mated female, 4–5 days old, was released into the common arm of the Y-tube and exposed to one of two odor combinations consisting of (a) clean air (blank) versus (b) a peach or pear twig. A total of 40 females per strain were tested against each odor combination, resulting in a total of 80 females per strain used. A new plant sample was used for every other individual tested. The fecundity of 40 additional females per strain was also assessed. Each female (newly emerged and virgin) was mated to a newly emerged virgin male from the stock population in a separate plastic box (6 cm× 4 cm× 2 cm). Water was provided ad libitum. All plastic boxes were kept in a controlled climate chamber (Conviron Ltd., Winnipeg, MB, Canada) with L:D 16:8,

60 % RH, and 24 °C. After 3 days, males were removed and all females were allowed to continue laying eggs in their respective boxes until death. The total number of eggs laid per female (and their viability) was recorded.

Statistical analyses

Results from the Y-tube olfactometer bioassays were analyzed for preference (percentage of responding females that made a choice for either the plant material or the control) and responsiveness (proportion of females that made a choice). Chi-square tests were carried out to test the null hypothesis of no odor preference for a particular plant odor versus the air control (R, version 2.10.1). Females that did not make a choice were excluded from these analyses. Then, unpaired-sample t tests were carried out to compare responsiveness across all possible combinations within each female group tested (SPSS, 16.0). When relevant, the alpha value for each comparison was adjusted downward using the Benjamin and Hochberg procedure to correct for false discovery rates (type 1 errors) (Verhoeven et al. 2005).

Female olfactory attraction from the different full-sib and half-sib families used in the heritability studies was analyzed for preference and responsiveness as described above. Since female fecundity values were not normally distributed, a Kruskal–Wallis test was conducted to test for differences in fecundity across full-sib and half-sib families (JMP 9). To test for a correlation between female odor preference and fecundity, a pair-wise Pearson correlation analysis was conducted (JMP 9.0). The heritabilities of both female odor preference and fecundity were then calculated. Female odor preference, scored in two categories, was treated as a threshold trait assuming an underlying continuous variable (Falconer and Mackay 1996), while female fecundity, measured as number of eggs produced during life span, was treated as a continuously distributed trait.

The heritabilities of female odor preference and fecundity as well as the genetic, residual and phenotypic correlations between the two traits were estimated using the program THRGIBBS1F90 (Tsuruta and Misztal 2006; http://nce.ads.uga.edu/∼ignacy/newprograms.html accessed June 30, 2011). Additive genetic and residual variances and covariances were estimated with an animal model assuming unrelated parents of the observed insects, i.e. unknown parent groups, and applying a Bayesian analysis using a Gibbs sampler (Van Tassell et al. 1998). Twenty thousand rounds of iterations were performed. After a burn-in period of 1,000 rounds, estimates of heritabilities and correlations were computed from the results for the variance and covariance components of every 100th round, and from these, final estimates and their standard deviations were obtained.

Results

Heritable component of female olfactory attraction and fecundity

Mated females from the different families varied strongly in their phenotypic olfactory preference and responsiveness (Fig. 1) and in mean phenotypic fecundity values (Fig. 2). Two half-sib families (1A/B and 2A/B) consistently showed statistically significant preference for the plant odor over clean air (blank), whereas preference for the plant odor among the remaining eight half-sib families (3A/B to 10A/B) was irregular. In three cases (half-sib families 3A/B, 4A/B and 10 A/B), one full-sib family significantly preferred the plant odor (3B, 4A and 10A) whereas the other full-sib family did not show a significant preference for either odor choice (3A, 4B and 10B), and the opposite (preference for blank vs. no preference) applied to four cases (5A/B, 6A/B, 7A/B and 9A/B). In one case (8A/B), neither of the two full-sib families showed any significant preference (Fig. 1). Female responsiveness also varied significantly across families ($t = 1.81$, $df = 18$, $P = 0.04$), with percentages of females making a choice ranging from 61 to 100 (Fig. 1). In addition, female fecundity also varied significantly across full-sib and half-sib families (Kruskal–Wallis test statistic = 62.06,
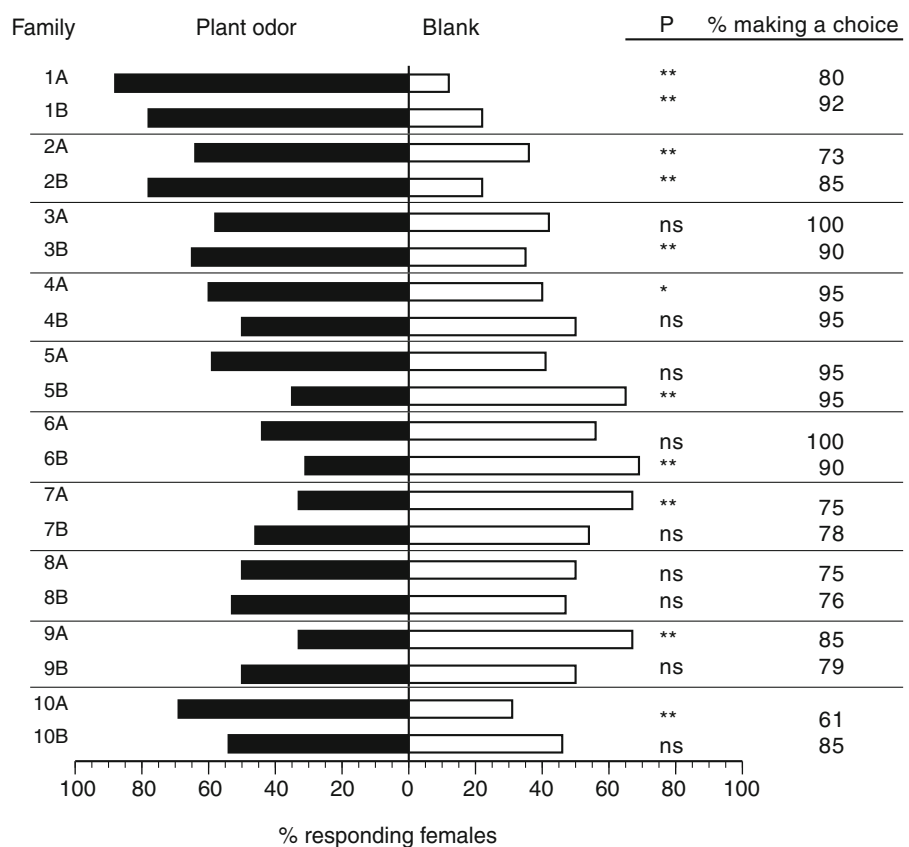
$df = 19$, $P \leq 0.01$), with mean values ranging from 144 to 201 eggs/female (Fig. 2). The phenotypic correlation between family mean values for female olfactory preference and fecundity was negative and significant (Pearson's correlation $r = -0.50$, $P = 0.04$, $N = 20$) (Fig. 3).

The estimates of heritability (h^2) for female olfactory preference and female fecundity amounted to 0.37 ± 0.17 and 0.32 ± 0.13, respectively, with these two traits showing a strong negative genetic correlation between them ($r_g = -0.85 \pm 0.21$) (Table 1). The negative genetic correlation was counterbalanced by an estimated positive residual correlation ($r_e = 0.46 \pm 0.20$). Thus, the resulting phenotypic correlation was nearly zero ($r_p = -0.01 \pm 0.09$) (Table 1).

Comparison of female olfactory attraction and fecundity between two strains differing in the number of generations maintained in the laboratory

The negative genetic correlation between olfactory preference and female fecundity was corroborated empirically when comparing the olfactory responses and fecundity of two oriental fruit moth strains maintained in the laboratory for short-term (less than 10 generations) or for long-term (over 70 generations), respectively. Females from the short-term reared strain significantly preferred plant odors over

Fig. 1 Olfactory responses of mated oriental fruit moth females from different full-sib and half-sib families to host plant (peach) odor versus clean air (blank) in Y-tube olfactometer bioassays. Families assigned the same number were sired by the same male, which was mated with two females each, yielding the full-sib families A and B, the members of which are related as half-sibs. $N = 20$ females/full-sib family. P values based on Chi-square tests. $P > 0.05 = ns$
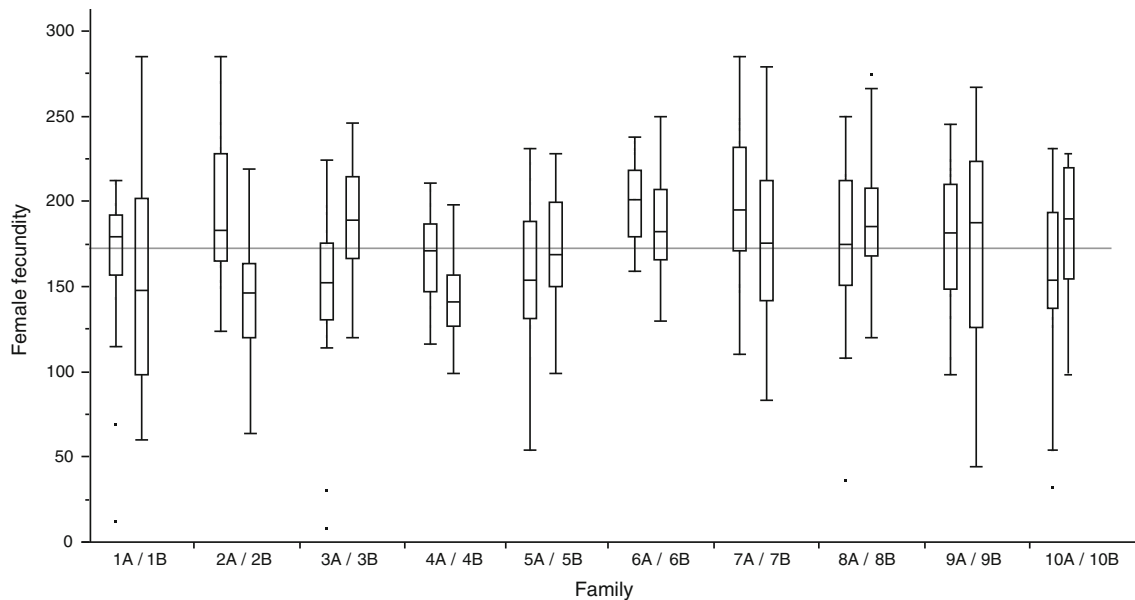
Family	P	% making a choice
1A	**	80
1B	**	92
2A	**	73
2B	**	85
3A	ns	100
3B	**	90
4A	*	95
4B	ns	95
5A	ns	95
5B	**	95
6A	ns	100
6B	**	90
7A	**	75
7B	ns	78
8A	ns	75
8B	ns	76
9A	**	85
9B	ns	79
10A	**	61
10B	ns	85

Fig. 2 Variability in fecundity values (median, quartiles and data range) of mated oriental fruit moth females from different full-sib and half-sib families (as detailed in legend to Fig. 1). *Dots* indicate outliers outside 1.5× inter-quantile range
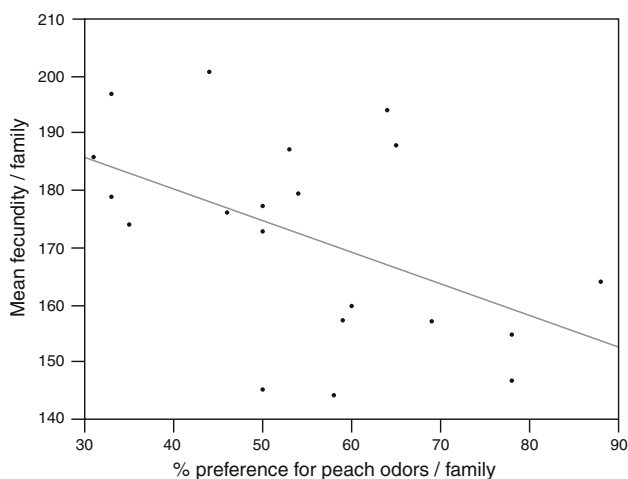

Fig. 3 Phenotypic correlation between percentage of females preferring host plant odors per full-sib family and mean female fecundity per full-sib family. Each family ($N = 20$) is represented by a *dot*. The *gray line* represents the linear fit of the regression model ($R^2 = 0.251$, slope $= -0.45 \pm 0.18$, $F = 6.05$, $df = 19$, $P = 0.024$)

clean air, whereas females from the long-term reared strain were not able to discriminate between these odors (Fig. 4). These results were consistent irrespective of whether the plant odor originated from the moth's primary host peach, or its secondary host pear. Female responsiveness did not differ significantly across the two strains, again irrespective of plant odor source ($t = 0.25$, $df = 2$, $P = 0.83$) (Fig. 4). Remarkably, mean female fecundity values for the strain maintained for short-term (172.03 ± 3.92 eggs/female) were significantly lower compared to those for the strain

maintained long-term (226.9 ± 8.94 eggs/female) (Kruskal–Wallis test statistic $= 18.20$, $df = 1$, $P = < 0.01$).

Discussion

Our genetic analyses revealed, for the first time, a significant genetic component in the odor-guided host plant location behavior in an herbivore moth species. Furthermore, they provided the first empirical evidence for a strong genetic trade-off between female insect attraction to host plant odors and fecundity. The implications of these findings are explored below from an evolutionary and ecological perspective.

Heritability and genetic correlation estimations

Heritability estimates based on a full-sib/half-sib design indicate a considerable genetic basis for innate attraction of females to host plant odors and for females' fecundity in the oriental fruit moth. The heritability estimates of olfactory attraction in this herbivore species was much higher than in the only other herbivore insect species with a heritability estimate available, the fruit fly *Drosophila melanogaster* (Meigen) (Mackay et al. 1996), and it was similar to that of two carnivore arthropod species, the parasitoid wasp *Cotesia glomerata* L. (Gu and Dorn 2000) and the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Margolies et al. 1997) (Online Resource 1). The heritability estimate of fecundity calculated for the oriental fruit moth lay in a higher or in a similar range as most available estimates for this trait

Table 1 Heritability (h^2) and correlation estimations and standard deviations (SD) for olfactory preference and fecundity of mated oriental fruit moth females. The calculations were based on a full-sib/half-sib design

Parameter	Estimate	SD
Heritability of female olfactory preference	0.37	0.17
Heritability of female fecundity	0.32	0.13
Genetic correlation (r_g) between female olfactory preference and fecundity	−0.85	0.21
Residual correlation (r_e) between female olfactory preference and fecundity	0.46	0.20
Phenotypic correlation (r_p) between female olfactory preference and fecundity	−0.01	0.09

Fig. 4 Olfactory responses of mated oriental fruit moth females from two strains to host plant (peach or pear) odors versus clean air (blank) in Y-tube olfactometer bioassays. The two strains were reared under the same laboratory conditions for short-term (grey bars) and long-term (black bars), respectively. N = 30 females/odor combination tested. P values based on chi-square tests. P > 0.05 = ns
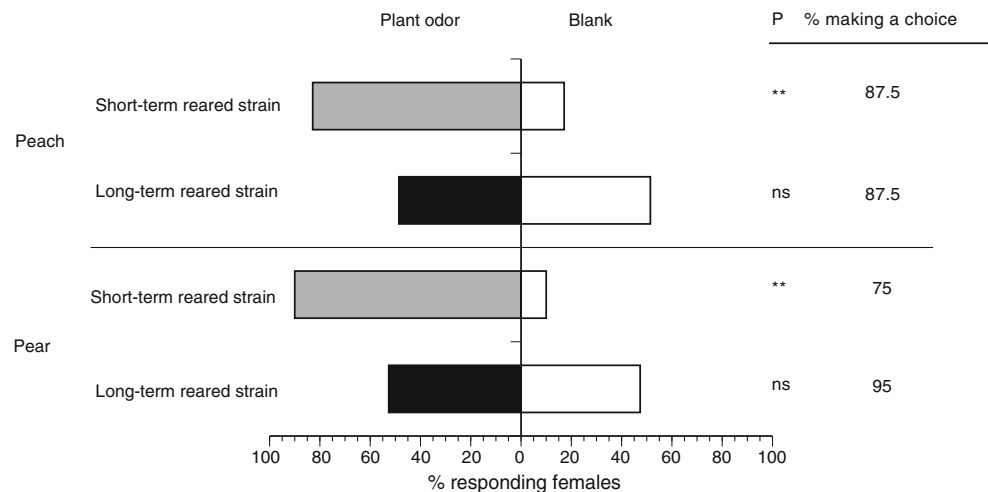
across the animal kingdom including arthropods (Online Resource 1) and higher animal taxa, such as fishes (e.g. Lester et al. 1988), birds (e.g. Sheldon et al. 2003; Teplitsky et al. 2009) and mammals (e.g. Olivier et al. 1998; Kruuk et al. 2000).

In our full-sib/half-sib experiment, which considered female olfactory preference and fecundity simultaneously within one single generation, the negative genetic correlation between these two traits was counterbalanced by an estimated positive residual correlation. This residual correlation is due to non-additive genetic factors, and mainly to uncontrolled environmental variation (Falconer and Mackay 1996), influencing here both traits in the same direction. Nutritional and climatic factors, which are at least partly under experimental control, are the most common external causes of environmental variation (Falconer and Mackay 1996). Although in the present study nutritional factors were standardized by using the same artificial diet for all families and individuals, and key climatic factors were kept under control in a climate chamber, additional uncontrollable factors, e.g. different nutrient uptake by families or individuals, could have affected our results.

Genetic basis of odor guided behavior

Studies on the genetic basis of innate host plant selection by herbivore insects have been virtually limited to post-

alighting behaviors (Carrière and Roitberg 1995; Jallow and Zalucki 1996; Hägele and Rowell-Rahier 2000), and have largely neglected pre-alighting behaviors, in particular odor-guided attraction to host plants. To our knowledge, previous evidence for a genetic basis of odor-guided herbivore attraction is confined to species of the order Diptera. In the apple fruit fly *Rhagoletis pomonella* (Walsh), fruit odor discrimination among host races and sibling species has a genetic basis that involves cytoplasmic-nuclear gene interactions (maternal effects) and epistasis (Dambroski et al. 2005). In the fruit fly *D. melanogaster*, variation in response to synthetic odorants, particularly esters, aldehydes and alcohols, exists across populations (Fuyama 1976; Alcorta and Rubio 1989; Mackay et al. 1996), and a large component of this variation seems to be controlled by genes located in the right arm of the second chromosome (Fuyama 1978). Successful artificial selection for increased or decreased olfactory responses to ethanol, acetaldehyde, and acetic acid was achieved (Hoffmann et al. 1983). Interestingly, selection for ethanol did not result in a change in response to acetaldehyde (and vice versa), suggesting that there are at least two independent genetic systems involved. Our investigation is the first to present quantitative genetic data documenting heritability for odor-guided female attraction to host plants in a non-dipteran herbivore insect.

Aside from herbivore insects, a genetic basis for innate odor-guided behavior has been demonstrated for two parasitoids of the order Hymenoptera. The innate flight responses of isofemale lines of the parasitic wasp *C. glomerata* to plant and host-related odors were subject to genetic variation (Gu and Dorn 2000), which was confirmed by bidirectional selection (Wang et al. 2003; Wang et al. 2004). Parent-offspring analysis indicated a heritable component in the responses to fruit odors by different strains of *Leptopilina boulardii* (Barbotin, Carton & Keiner-Pillault), a parasitoid of *D. melanogaster* (Campan et al. 2002). In animal taxa beyond insects, a genetic basis for innate responses to prey-related odors has been found in the predatory mite *P. persimilis* (Margolies et al. 1997; Jia et al. 2002) and in the garter snake *Thamnophis elegans* (Baird & Girard) (Arnold 1981).

Maintenance of genetic variation in female olfactory attraction within a population. Mechanisms and adaptive significance

The findings of our study indicating a considerable genetic polymorphism in olfactory attraction of a female herbivore insect raise important questions on the processes that influence the maintenance of this genetic variation in field populations. In a system in which insect individuals use plant odors to discriminate between host and non-host plants, selection should favor insect genotypes which yield phenotypes that respond strongly to these odors. Plant odors, however, are not precise blends, as they vary quantitatively and/or qualitatively with plant genotype and phenological stage (Dudareva and Pichersky 2000; Hern and Dorn 2003), as well as with daily and seasonal variations in environmental conditions (Vallat et al. 2005; Rodriguez-Saona et al. 2011). Thus, genetic variation among individual females in attraction to host plant odors should have an adaptive significance (Najar-Rodriguez et al. 2010, 2011), as the temporally and spatially fluctuating plant signals should favor the persistence of different olfactory genotypes.

The oriental fruit moth is an oligophagous and a multivoltine insect that uses peach as primary and pome fruits such as apple and pear as secondary hosts (Rothschild and Vickers 1991). Host shifts of this herbivore from peach to pome fruits have been observed with progressing season (Hughes and Dorn 2002; Kovanci et al. 2004). The existence of different olfactory insect genotypes may facilitate host shifts because a single genotype could not produce an average phenotype that is optimal in all environments across space and time (Gillespie and Turelli 1989; Wang et al. 2004; Nachappa et al. 2010). In fact, habitat heterogeneity has been hypothesized to likely cause maintenance of high genetic variation in foraging traits that are associated with efficient prey location and use in the predatory mite *P. persimilis* (Nachappa et al. 2010). In evolutionary terms, oriental fruit moth populations comprising different olfactory genotypes would be better adapted to specific olfactory environments at any given time.

Phenotypic plasticity, i.e. differential expression of phenotypes in response to environmental variation, has also been suggested as a strategy that allows organisms to deal with changing environmental conditions (e.g. Sambandan et al. 2008; Hunt et al. 2011). However, for the oriental fruit moth as well as for other short-lived insect herbivores, the influence of genetic variation would outweigh the importance of plasticity because the lifespan of the insects is short in comparison to the time scale for dynamic changes in plant volatile emission. Thus, within an individual's lifespan the environment could be considered as relatively homogeneous and stable, but across generations it is likely to change considerably, resulting in variable patterns of natural selection over time (Nachappa et al. 2010).

Ecological and evolutionary consequences of a trade-off between female odor preferences and fecundity

Trade-offs between behavioural and life-history traits in animals might have an adaptive significance as well as ecological consequences (e.g. Hughes et al. 2003; Roff and Fairbairn 2007; Evans 2010). The trade-off between the genetic components of odor attraction and fecundity in the oriental fruit moth, in particular, might be relevant when mated females undertake flights between non-contiguous host plant patches (Hughes and Dorn 2002; Torriani et al. 2010). Enhanced olfactory searching ability of the oriental fruit moth females would strongly facilitate efficient host plant location. However, the enhanced olfactory ability in these females would imply a fitness cost in terms of reduced offspring, as suggested by the negative value for the genetic correlation between female olfactory preference and fecundity. In the parasitic wasp *C. glomerata*, an artificially selected high-olfactory responding strain foraged more efficiently for hosts over a wider habitat area compared to the low-olfactory responding strain (Wang et al. 2004). Although a genetic trade-off between female olfactory attraction and fecundity has not been previously reported for other herbivore insects and has also not been found in *C. glomerata* (Wang et al. 2004), a negative phenotypic correlation between the two traits has been documented for the predatory mite *P. persimilis* (Jia et al. 2002), suggesting that such trade-off might be more widespread across arthropods.

A trade-off between dispersal and fecundity, i.e. between another key behavior and fecundity, has been

shown across different insect taxa, including several lepidopteran species (Gu and Danthanarayana 1992; Zera and Denno 1997; Hughes et al. 2003; Gu et al. 2006), some of which are from the same family (Tortricidae) as the oriental fruit moth. The negative correlation between dispersal and fecundity was attributed to a trade-off between internal resource allocation, in particular energy (Zera and Denno 1997). Thus, the genetic trade-off between olfactory attraction and fecundity documented in this study for the oriental fruit moth could imply similar conflicting resource allocation. Field populations of the oriental fruit moth are known to be predominantly relatively sedentary being composed of a small proportion of females qualified as long fliers (with individuals being able to make a single flight of greater than 1 km) and a greater proportion of short fliers (Hughes and Dorn 2002). Thus, if a positive correlation between olfactory responses and dispersal holds for the oriental fruit moth, and if dispersal is also negatively correlated with fecundity in this species, it is then possible that long-flier females that colonize new habitats have high olfactory abilities but low fecundities compared to short flier females that stay near natal patches. Long fliers might leave deteriorating host plant patches to colonize more nutritious stands located elsewhere, thus compensating partially for their reduced fecundity by providing their offspring with food of higher nutritional value.

A closer understanding of the relationship between female olfactory attraction and fecundity in the oriental fruit moth will require further elucidation of the genetic basis of the trade-off, including the identification of genes that regulate the two traits, their gene-to-gene interactions (e.g. pleiotropy and/or epistasis) and their gene-to-environment interactions, as well as comprehension of their variational properties (e.g. Lavagnino et al. 2012). Linkage-based mapping techniques such as quantitative trait locus (QTL) could be used for this purpose. Correlation studies between female olfactory preferences and other behavioral traits (e.g. dispersal and thermal preference) or life-history traits (e.g. female oviposition preference, mating and/or longevity) that are linked to successful host plant location could also shed further light into the ecological implications of correlations between behavioral and life-history traits.

Corroboration of a trade-off between female olfactory attraction and fecundity

The genetic trade-off between female olfactory preference and fecundity found in our quantitative genetic studies was empirically corroborated and became phenotypically apparent when comparing the olfactory preferences and the mean fecundity values between individuals from the short- and the long-term laboratory-reared strains. The two strains shared a common geographical origin and were reared under the same controlled environment. Thus, females with higher fecundity but with lower olfactory discrimination abilities apparently were inadvertently selected in the course of the more than 70 generations of maintenance in the laboratory. The differences in odor attraction between these two strains could be further used to examine the neurophysiological mechanisms and the ecological consequences of decreased olfactory abilities in the oriental fruit moth.

In conclusion, our results reveal significant genetic variation for female olfactory attraction and fecundity in the oriental fruit moth, an important invasive oligophagous species. Ecological implications of this finding might be relevant beyond the herbivore species studied, shedding new light into the relationship between host plants, with their volatile emissions, and insect populations comprising different genotypes. The oriental fruit moth challenges fruit production worldwide, and a promising strategy to manage such pest populations is the attraction of females to specific odors (Hern and Dorn 2004). Thus, our results would also have practical implications, as they provide important baseline information for forecasting the long-term success of insect pest management strategies involving behavioral manipulation with plant volatiles, e.g. insect attraction to odor-baited traps. Given that high-olfactory responding females would be trapped at a higher rate than low-olfactory responding (but highly fertile) females, such selection pressure would eventually lead to a decreased percentage of females attracted to traps. Such inadvertent selection could impede the success of mass trapping-based control, which requires a high percentage of insects being attracted to the odor source, but would have comparatively little implications when traps are merely used to monitor the incidence and timing of female moth occurrence. Furthermore, as corroborated empirically in the present study, populations maintained under laboratory conditions, which exhibit selection pressure for fecundity, might be prone to lose genotypes with high olfactory responses to host plant volatiles. Thus, the genetic trade-off detected in this study between female olfactory attraction and fecundity for a lepidopteran species highlights the inherent constraint for simultaneous genetic improvement of these traits.

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