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Genetic Variability of Conditioned Probing Responses to a Fruit Odor in *Leptopilina boulardi* (Hymenoptera: Eucoilidae), a *Drosophila* Parasitoid

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The genetic variability of odor-conditioned probing behavior was investigated in a population of *Leptopilina boulardi*, a parasitoid of *Drosophila* larvae. Ovipositor probing is the final step of host location, leading to the discovery of host larvae. It can be triggered by an odor previously experienced during an oviposition as a result of associative learning. This study was based on the analysis of female probing performance over two generations of isofemale lines (using both mother–daughter regressions and one-way analysis of variance). Individual performances of the conditioned response to the odor were characterized by (1) the latency (*i.e.*, the time elapsed between the onset of the odor delivery and the start of the probing response), (2) the duration of the first probing phase, and (3) the total probing duration. Results suggested that the variability of two characters, the latency and the duration of the first probing phase, were under a genetic control in the studied population. This work is the first contribution to quantify the genetic component of this variability.

KEY WORDS: *Leptopilina boulardi*; parasitoid; genetic variability; olfactory learning; searching behavior.

INTRODUCTION

The reproductive success of female parasitoids depends on their ability to find and select suitable hosts in a changing and diversified environment. Host finding results from successive behavioral steps, from orientation toward a potential host habitat to the actual host location (Vinson, 1976). Host finding is mostly based on the recognition of chemical cues (Vet and Dicke, 1992; Tumlinson *et al.*, 1993). It has been demonstrated in a number of species that females can learn chemicals associated to the presence of suitable hosts and that resulting behavioral changes are crucial for the adaptation to host resources (Lewis and Tumlinson, 1988; De Jong and Kaiser, 1991; Turlings *et al.*, 1993; Vet *et al.*, 1996). Such associative learning ability has likely been progressively settled by natural selection leading to an increase in female fitness in the course of generations. In return, a progressive settlement of such learning should depend on individual differences determined by genetic variation on which natural selection could act.

Genetic variability of abilities for associative learning has been demonstrated for taste in the blowfly *Phormia regina* (McGuire and Hirsch, 1977; Tully and Hirsch, 1982) and for odors in the

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honeybee *Apis mellifera* (Brandes, 1988, 1991; Bhagavan *et al.*, 1994). These studies were based on bidirectional selection of odor learning performances, measured by conditioned proboscis extension. They were facilitated by the possibility of measuring learning performances of males or by the use of parthenogenetic bees [*A. mellifera capensis* (Brandes, 1988, 1991)]. In the case of parasitoid insects, we are interested in the genetic variability of odor learning involved in a host location behavior performed by females. One of the quantitative genetic methods available for such studies involves the use of isofemale lines (*i.e.*, the progeny derived from a single, randomly inseminated female), which allows the evaluation of the genetic variability of a trait when the genome is not known (Hoffmann and Parsons, 1988). Estimates of the relative proportions of genetic and environmental variances in populations can be obtained from variability within and between isofemale strains calculated using an analysis of variance. This method has been successfully employed with hymenopterous parasitoids showing a haplodiploid reproduction system and, particularly, to investigate the genetic variability of reproductive behavioral traits (Chassain and Boulétreau, 1987; Carton *et al.*, 1989; Wajnberg *et al.*, 1989; Delpuech *et al.*, 1993; Bruins *et al.*, 1994).

Odor learning involved in host location has been well studied in *Leptopilina boulardi* (Hymenoptera: Eucoilidae), a parasitoid of frugivorous *Drosophila* larvae. Through associative learning, females remember odors that they perceived during oviposition. They consequently exhibit conditioned orientation and ovipositor search of a substrate (probing) in response to the learned odors (De Jong and Kaiser, 1991; Kaiser and De Jong, 1995; Kaiser *et al.*, 1995), a behavior which should increase the probability of finding a host larva. We developed a procedure for the odor conditioning of the probing behavior to quantify individual learning performances (Kaiser *et al.*, 1995). Systematic probing (93%) in response to the conditioning odor (banana) was observed after associative conditioning (*i.e.*, after the odor had been experienced while ovipositing). Naive females without odor or oviposition experience exhibited a low rate of response (23%). The percentage of females responding to the odor was also increased but to a lesser extent after oviposition experience alone (67%) or preceded by exposure to banana odor (52%). This indicated that responses to banana odor after the associative conditioning treatment were evoked partly by sensitization. So probing activity appears to be much modulated by the wasp's experience. As oviposition is always associated with the perception of the fruit odor under natural conditions, we decided to quantify the genetic variability of responses following associative conditioning to banana odor, within a *L. boulardi* population. We examined two successive generations of isofemale lines from a laboratory population using mother–daughter regressions and sib analysis.

MATERIALS AND METHODS

Rearing of the Isofemale Lines

The *L. boulardi* strain used in this study originated from parasitized *Drosophila melanogaster* pupae collected from prickle pear (*Opuntia*) in Tunisia (Nasrallah) in 1986. Since then, it has been maintained on its sympatric host in population cages (strain G464; Y. Carton). Thirty lines were started, each from a mated female at the grandmother generation. Females of the mother generation were mated with their brothers, to increase the genetic relatedness between females of the mother and the daughter generations. At the mother generation, one female per grandmother was randomly selected and evaluated for its learning ability. It was then isolated in a rearing tube. At the daughter generation, seven or eight females per mother were randomly taken for the same evaluation.

The following rearing procedure was used to produce the mother and daughter generations: Females were allowed to parasitize larvae of the *Rosy 295* mutant of *D. melanogaster* [a mutant selected for a high parasitization rate (Carton and Frey, personnal communication)] which had been fed on an axenic diet (cornmeal, dead pulverulent yeast, sugar, and a fungicide). To minimize the olfactory experience of parasitoids before experiments, pupae of parasitized *Drosophila* were collected, washed in 5% bleach (also to prevent the development of fungi), rinsed in water, and stored at 25°C and LD 12:12 in tubes containing agar–agar and honey. Females were mated as soon as they emerged. After emergence of the parasitoids, tubes were stored at 17°C. They were distributed into two photoperiods with a 0900–2100 light cycle and a 1300–0100 light cycle to homogenize the level of activity of females observed in the morning and in the afternoon. Experiments were conducted on 5- to 7-day-old, mated females, 4 h from the beginning of the light cycle, at 20–22°C.

Conditioning and Testing Protocol

A single female was introduced into a device allowing simultaneous or separated presentation of larvae and an odor (see below). It consisted of a ring of agar–agar (internal diameter: 6 mm) closed with a nylon bottom and a perforated acetate lid (caplet). A continuous airflow (750 ml/min), which could be odorized, arrived from underneath the device, with a gap of 2 mm separating the air outlet and the caplet. The actual flow through the caplet was lower than 750 ml/min and did not disturb the wasp (for details see Kaiser *et al.*, 1995).

Odor Source

Two glass capillaries (1 cm long, 1.56 mm in internal diameter) with one extremity sealed with wax were filled with banana extract (Haarman and Reimer, St-Ouen-l'Aumone, France) and served as the odor source for conditioning and testing (Kaiser *et al.*, 1995).

The protocol included three consecutive phases (Fig. 1).

The conditioning phase consisted of three odor-associated ovipositions (about 20 second-instar larvae had been deposited on the ring of agar just prior to the experiments). The total duration of these conditioning trials was recorded.

The second phase, which followed immediately afterward, was a 1-h intermediate phase, without larva and odor, that allowed the female to stop probing the agar, an excitatory response often seen as a consequence of oviposition (Kaiser *et al.*, 1995).

The third phase was a test of the response to the conditioned odor. After a 1-min observation for checking the absence of excitatory probing, the conditioned odor was delivered into the continuous airflow for 20 s (mean duration of an oviposition). Conditioned probing behavior was observed until a female stopped probing for at least 1 min and up to 5 min if it did not stop. This behavior was characterized by (1) the latency of the probing response following the onset of odor delivery, (2) the duration of the first probing phase, and (3) the total probing duration.

As shown previously, the conditioned response to the banana odor was due partly to sensitization through oviposition experience by itself when tested 12 min after the conditioning phase (Kaiser *et al.*, 1995). To check for the importance of sensitization when the test was performed 1 h after the conditioning phase, a control group where females oviposited three times under the continuous nonodorized airflow was tested.

Data Analysis

The frequencies of conditioned probing responses among females from the 30 lines were first compared using a χ^2 test. Then, the genetic variability of the individual response parameters to the conditioning odor was analyzed using both mother–daughter linear regression and one-way ANOVA (line effect).

Spearman rank correlation was also calculated between the parameter of the conditioning phase (total duration of the three ovipositions) and each parameter of the probing response to the conditioned odor (latency, duration of the first probing phase, and total probing duration) in order to check for an eventual relationship between the variability observed during the conditioning phase and that of the conditioned odor response. The correlation between parameters showing significant genetic variability was also calculated.

RESULTS

Frequencies of Conditioned Probing Responses

In the mother generation, only 1 female of 30 did not exhibit the conditioned response. In the daughter generation, the frequencies of responses were not significantly different between lines ($\chi^2 = 23.7$, df = 29, NS) and varied between 2 of 7 (29%) and 7 of 8 (88%). The overall percentage of response in the daughter generation was 69%.

The control group showed a lower rate of probing responses to the banana odor (43%; $n = 30$) compared to the overall percentage of responding daughters ($\chi^2 = 8.05$, 1 df, $p < .01$) and to the 97% of responding mothers.

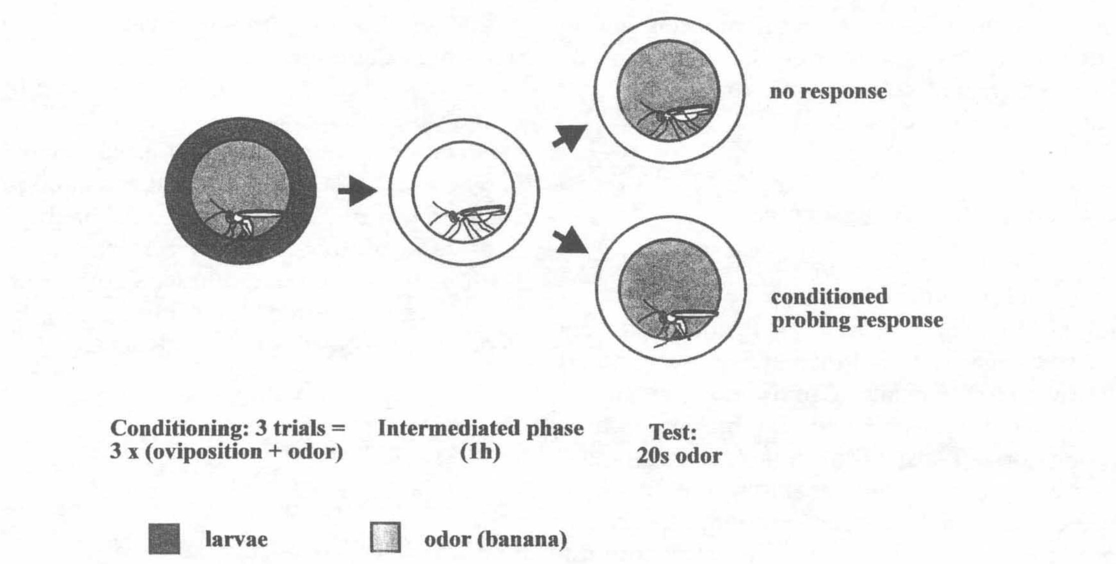

Fig. 1. Conditioning and testing protocol (see text for explanations). A female is introduced into a device allowing simultaneous or separated presentation of larvae and an odor: it consists of a ring of agar–agar (internal diameter: 6 mm) closed with a nylon bottom and a perforated acetate top. An airflow which can be odorized arrives continuously from underneath the device (from Kaiser *et al.*, 1995).

Analyses of the Individual Parameters of the Conditioned Probing Response

Only the females that responded to the odor were considered, so the latency could be measured. This procedure could not induce a line effect because the percentage of conditioned responses did not differ among lines.

The mother–daughter regression on the duration of the first probing phase was significant at the 5% probability level and the value of r^2 indicated that 12.6% of the variability in daughter values was explained by mother values (Fig. 2b). For the other parameters, the regression coefficient was not significantly different from 0 (Figs. 2a and c).

The analysis of variance showed a significant isofemale line effect on the latency of the conditioned response (Table I). Mean latencies varied from 6 ± 2 (SE) to 41 ± 13 s. There was no significant line effect on the other parameters (Table I). Mean values of the first probing phase varied from 5.2 ± 2.1 to 42.2 ± 10.9 s. Mean values of the total probing duration varied from 25 ± 16 to 104 ± 34.3 s.

These results suggest that, in the population analyzed, there was a significant genetic component of the phenotypic variability both in the latency of the conditioned response (Table I) and in the duration of the first probing phase (Fig. 2b). Figure 3 shows that there was no significant correlation between these two behavioral traits among the 30 lines studied.

Finally, there was no significant correlation between the total duration of the three ovipositions during the conditioning phase and any of the parameters of the conditioned response (latency, $r = .32$; duration of the first probing phase, $r = -.19$; total probing duration, $r = .07$). Therefore, the genetic variations observed in the conditioned response did not seem to be related to the phenotypic variation observed during the conditioning phase.

DISCUSSION

The lower but significant rate of responses to banana odor after oviposition experience compared to banana-associated ovipositions indicates that oviposition experience by itself sensitizes the wasps to the fruit odor and consequently contributes to the effect of associative conditioning. We chose to study the variability of responses after associative conditioning because females naturally smell the substrate odor when ovipositing. A resulting probing response to banana odor would facilitate the discovery of other host-infested patches within the explored fruit

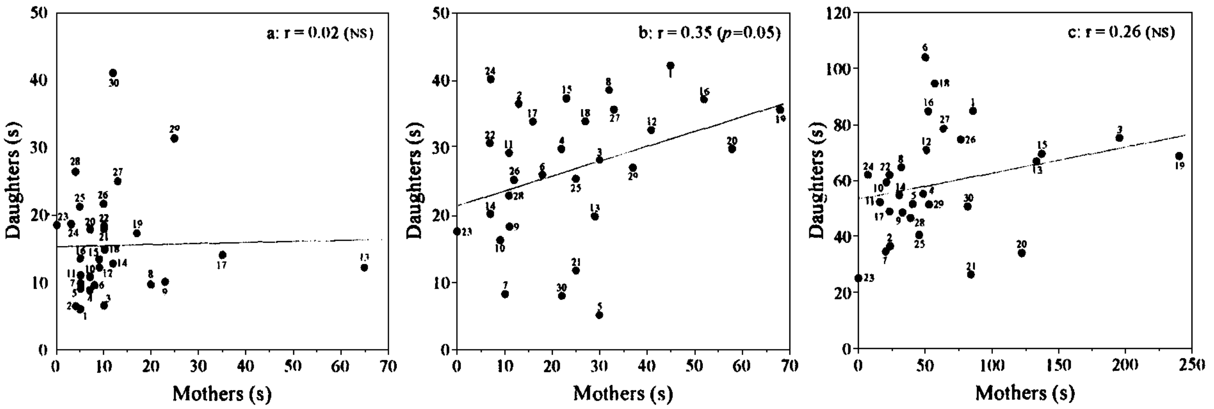

Fig. 2. Mother–daughter regression analyses of the values of (a) latency, (b) first probing duration, and (c) total probing duration. Mean values of the daughters of each line are plotted on the *y* axis. Lines are ordered according to increasing mean daughter values of latency.

Table I. One-Way Analysis of Variance on the Three Characters Measuring Individual Conditioned Probing Responses: Latency of the Response Following the Onset of Odor Delivery, Duration of the First Probing Phase, and Total Probing Duration Within 5 min of Observation Following the Onset of Odor Delivery

Character	Source	df	Mean square	*F* value
Latency	Between	29	265.93	1.72*
	Within	117	154.95	
	Total	146	177	
First probing	Between	29	467.68	NS
	Within	117	347.72	
	Total	146	371.56	
Total probing	Between	29	1678.8	NS
	Within	117	2510.05	
	Total	146	2344.94	

* $p<.05$.

(discrete distribution due to *Drosophila* oviposition restricted to wounds on the peel). Odors associated with oviposition experience also trigger orientation responses in *Leptopilina* and many species of parasitic wasps. This learned response increases the probability of discovering new host-infested sites by focusing females search toward substrates of which infestation is highly probable (i.e., review by Vet *et al.*, 1995). We demonstrated previously that *L. boulardi* females oriented to a fruit odor only when it had been perceived during oviposition experience, whereas mere oviposition had no effect on this behavior (De Jong and Kaiser, 1991). This proved that different physiological changes arise from both types of experience.

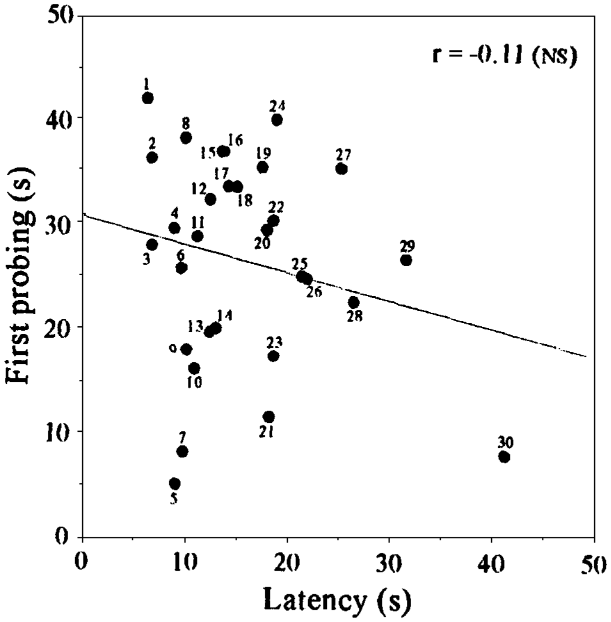

Fig. 3. Correlation between mean daughter values of latency and first probing duration. Lines are numbered as in Fig. 2.

Phenotypic variability of odor-conditioned behaviors had already been observed in *L. boulardi* (De Jong and Kaiser, 1991; Kaiser *et al.*, 1994) and was confirmed in this work. Here, we additionally searched for the genetic component of the observed variability of the odor-conditioned probing.

For the latency of the conditioned response and for the duration of first probing, mother–daughter regressions and analysis of variance on the daughter generation gave different results on the existence of genetic variability. This discrepancy has often been observed in studies of genetic variability in biological traits of parasitic wasps (e.g., Bruins *et al.*, 1994; Delpuech *et al.*, 1994). This could be due to the fact that the variability is not estimated using the same data in both methods. The analysis of variance compares the variability of values between and within families of daughters, whereas the regression is based only on the variability between families and over the mother and daughter generations. So the reliability of the estimates would be decreased by the fact that only one female is observed per family at the mother generation. The lack of significant mother–daughter regression may be attributed to a higher sensitivity of the latency to external factors. Moreover, from a statistical point of view, the probability of obtaining one significant result with two procedures by chance is much less than that of obtaining two nonsignificant results (9.5 and 90.25% respectively). This is why several authors, and ourselves, conclude a genetic component of the observed phenotypic variation when either parent–offspring regression or sib analysis gives a significant result.

The latency and duration of the first probing bout characterized the immediate response to the odor so they provide a good quantification of the conditioned response. As many females resumed probing after the first bout and could exhibit probing bouts over a few minutes, the total probing duration may reflect more of an excitatory state due to the oviposition experience during the conditioning period than a specific response to the conditioning scent. The latency and duration of the conditioned response may reflect more than an ability to be conditioned to an odor: they may be linked to a general ability to probe in response to a stimulus. However, it seems that the parameters of the spontaneous response to the odor of host-infested banana are not correlated with those of the conditioned response (Kaiser, unpublished data). Interestingly, there was no significant correlation between mean family values of the latency and the first probing duration. This suggests that the genetic variation in these characters may be governed by different genes.

In natural populations, the benefit of learning abilities may depend on the available host resources. Females that initiated probing more quickly or probed longer, in response to a fruit odor, should be favored in situations of competition or host-patches scarcity, respectively. Such a mechanism could explain the existence of significant genetic variability in wasp populations. Then the conservation of the genetic polymorphism in our experimental population could be related. to its adaptive value in response to environmental fluctuations.

This is the first investigation of the genetic variability of a learned response involved in the reproduction of a parasitic wasp. The analyses reveal that a genetic component contributes to the large phenotypic variability usually observed in such behavioral traits. Repeated analysis over larger samples is still needed to provide reliable estimates of this genetic component. With regard to the adaptable character of learning abilities, these findings could be considered for the improvement of parasitic efficiency in biological control programs.

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