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**Original article**

# Changes with age in olfactory conditioning performance of worker honey bees (*Apis mellifera*)

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**Abstract** – Proboscis extension reflex conditioning has often been used to study olfactory learning in the honey bee. However, only a few studies have explored the variability of this response relative to the age of the bees, and these studies have yielded different conclusions. We aimed to document the effect of age on the proboscis extension response by measuring three parameters in the experimental procedure: the level of spontaneous responses, the acquisition rate, and the resistance to extinction. The performance of the youngest bees (2-day and 4-day-old) differed from those of older individuals for the three parameters, which may be related to the ontogeny of the olfactory system. In older age-groups, only the resistance to extinction was found to vary with age. We discuss the possible relationships between age-dependent variations in the odour learning abilities and the division of labour in the colony.

**honey bee / *Apis mellifera* / conditioned proboscis extension / age effect**

## 1. INTRODUCTION

During its lifespan, a worker bee goes through a succession of different tasks, younger bees usually perform within-nest activities prior to becoming foragers. This behavioural ontogeny determines the division of labour in the colony, commonly known as age-polyethism (Michener, 1974; Oster and Wilson, 1978). The progression of the division of labour is not rigid and there is a great variability in the task ontogeny. The sources of variability include age, but other factors are involved such as the colony's state, the worker's genotype, and probably prior experience (for a general

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review on social insects, see Lenoir, 1987; on the honey bee, see Calderone and Page, 1988, 1991). The interactions between these different factors remain unclear, and the causal relationships between age and behaviour are still questioned. The claim that simple models, based on theories of self-organisation, can explain age-polyethism without any link between age and task performance (Tofts and Franks, 1992), have widely extended the debate. Nevertheless, such models appear to be too restrictive, and various mechanisms are probably involved in the process of behavioural ontogeny (Calderone, 1998). Undoubtedly, age is not the only explanation for polyethism, but it may play a major role in the division of labour.

Among the numerous tasks any worker has to perform, many of them require adaptative behaviours resulting from prior experience. Thus, the retinue behaviour of young worker attendants around the queen or swarming behaviour are based upon the ability to identify the queen, to determine her physiological state – virgin or mated, her age, and other factors (Boch et al., 1975; Winston et al., 1989; Free et al., 1990). This information is provided by the semiochemicals secreted and loaded on the queen body, which change according to the queen status (Crewe, 1982; Slessor et al., 1988; Apšegaite and Skirkevicius, 1994). The ability of the workers to adapt their behaviour to these changes might result from an olfactory learning process. Learning abilities are clearly involved in foraging behaviour, food source recognition being based on the memorisation of visual and olfactory cues (von Frisch, 1967; Menzel et al., 1993). Since olfactory learning is involved in different tasks occurring at various periods of the worker’s lifespan, we hypothesised that learning capabilities would vary according to the tasks, and consequently, with age.

The behavioural plasticity of bees has been widely studied in free-flying bees (von Frisch, 1967; Koltermann, 1969; Menzel, 1985) as well as in restrained individuals by recording the proboscis extension response (PER) (Takeda, 1961; Bitterman et al., 1983). This reflex can be trained in a classical conditioning procedure by presenting an odour in temporal association with a sugar reward. This procedure allows for good control of the conditioning parameters (Menzel et al., 1993; Sandoz et al., 1995) and of physiological variables (Mercer and Menzel, 1982; Erber et al., 1993). It has been used for studies on olfactory learning and memory processes (Erber, 1980; Menzel, 1984; Smith, 1991; Sandoz et al., 1995), on genetics of learning (Brandes, 1988, 1991) and on plant odour recognition (Pham-Delègue et al., 1993; Le Métayer et al., 1997).

Most studies based on PER conditioning used foragers of unknown age collected at the hive entrance. Few works have addressed the question of variability of olfactory learning performance according to age. Pham-Delègue et al. (1990) subjected different age groups to a training procedure, and showed that 12- to 18-day-old bees exhibited higher levels of olfactory conditioned responses than younger and older groups. This age range covers the period when the initiation of foraging tasks usually takes place under natural conditions (Seeley, 1982). Bhagavan et al. (1994), investigating the respective contribution of age, caste, and genotype on olfactory learning performance, found no effect of age from 5-day-old bees to the end of a bees’ lifetime, but they did not test younger bees. Moreover, as they observed a strong contribution of genotype on the variability in performance, they could not definitely exclude an age effect which might be hidden by the genotype effect. More recently, Ray and Ferneyhough (1997) studied olfactory conditioning among bees from one day after emergence to older stages. They found that younger bees, until 10 days of age, exhibited lower levels of conditioned response than older foragers.

The present work aimed to establish the ontogeny of olfactory learning abilities as measured in a PER procedure. Previous works on the influence of age on proboscis extension response have considered only the acquisition phase of the learning process. As the standard conditioning procedure (Bittermann et al., 1983) also allows for recording spontaneous responses and resistance to extinction, we analysed the effect of ageing on all three parameters. In addition, the odour stimulus used by Pham-Delègue et al. (1990) and Ray and Ferneyhough (1997) was geraniol. This volatile is not only a floral compound but is also a major component of the Nasonov pheromone (Pickett et al., 1980). As it is produced by all workers, in amounts which increase with age (Boch and Shearer, 1963), an effect of prior exposure on the conditioned response could not be excluded. In order to document a possible influence of the quality of the odour stimulus, we designed comparative experiments with geraniol and linalool, this second odour being of floral origin.

## 2. MATERIALS AND METHODS

### 2.1. Honey bees

The experiments were conducted on worker honey bees (*Apis mellifera* L.). Combs with pre-emerging brood were obtained between January and April 1998 from several hives maintained in a heated apiary. Emerging bees were collected from combs and caged in groups of 60–70 individuals of same age (Pain, 1966). Bees were provided with sugar and water ad libitum, and with pollen during the first eight days. They were maintained in an incubator (temperature: 33 °C, relative humidity: 55%, at dark). We tested groups of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20-day-old bees. To homogenise a possible effect of day, several bees from each age-group were tested every experimental day. This was repeated until the number of bees required for data treatment was reached (at least 20 bees were tested per age and per conditioning stimulus).

### 2.2. Conditioning of the proboscis extension

The stimulation conditions and the training procedures were adapted from the work of Bitterman et al. (1983) and are detailed elsewhere (Pham-Delègue et al., 1993; Sandoz et al., 1995). Bees were restrained individually in glass holders, then they were starved for 3 hours prior to the experiment. The proboscis extension reflex was tested on the first trial by contacting the antennae with a drop of 30% sucrose solution. Bees that did not exhibit the reflex were discarded.

The odour was delivered through a 1 cm diameter glass tube placed 2 cm in front of the head of the bee. The odour sample (10 µl) was soaked on a filter paper strip inserted in a Pasteur pipette cartridge. The vapour from the cartridge was delivered through an airstream (2.5 ml/s) which was then injected in the main airflow (50.5 ml/s). Comparative experiments were conducted with four different conditioning stimuli, either linalool (Sigma, 95–97%) or geraniol (Mallinckrodt, 99%), pure or at a concentration of $10^{-3}$ vol./vol. in hexane. When using a diluted stimulus, the solvent was let to evaporate for 30 s before inserting the filter paper in the cartridge.

Prior to each trial, the bee was placed in a clean airflow for 15 s in order to become familiarised with the mechanical stimulation and to the experimental context. Bees were then subjected to three conditioning trials followed by five extinction trials, with 15 min inter-trial periods. On each conditioning trial, the odour stimulus was presented for 6 s. In the last 3 s of the odour delivery, the antenna was contacted with a sucrose solution (30% w/w) and the extension of the proboscis was rewarded by the uptake of a drop of sucrose solution. On each extinction trial, the odour stimulus was delivered for 6 s with no sugar stimulation of the antenna and no reward delivery.

The responses of the worker bees were recorded at three trials within the experimental procedure:

(1) Spontaneous response (proboscis extension) to the odour was recorded during the first 3 s of the first trial, i.e. before the conditioning procedure per se. Bees that responded on this initial trial were discarded from the following acquisition procedure, since it would be impossible to distinguish bees responding spontaneously from those being conditioned.

(2) The extent of acquisition was recorded on the fourth trial, i.e. just after the end of the conditioning period. Bees that were not adequately conditioned at the end of this acquisition period were discarded from the following extinction procedure, in order to compare groups which displayed equivalent conditioning performance.

(3) Extinction was recorded on the last trial of the experimental procedure.

### 2.3. Statistical analyses

A comparison of the responses obtained with pure and diluted odour stimuli, and the comparison of the results obtained with geraniol and linalool, were analysed using a global $\chi^2$-test according to Cochran's rule (Cochran, 1954). The proportion of responses relative to age were compared to a theoretical uniform distribution using a homogeneity $\chi^2$-test. When the distribution was found to be non-uniform, two-by-two comparisons of the responses at different ages were undergone using a $\chi^2$-test with 1*df*. To ensure that the experiment error rate was $\alpha = 0.05$, we carried out each comparison according to the Dunn-Sidák method (Sokal and Rohlf, 1995) at a critical probability of $\alpha' = 1 - (1 - \alpha)^{1/k}$ where k was the number of intended tests. Therefore, the significance level was 0.0014 as we compared two-by-two the responses among ten age-groups. When conditions of application of the $\chi^2$-test were not fulfilled according to the Cochran's rule, the Fisher's exact method was used (Scherrer, 1984).

## 3. RESULTS

A comparison of data obtained with either pure or diluted ($10^{-3}$ vol./vol.) odour stimuli led to non-significant differences. Therefore, the responses obtained with the two concentrations of the same odour were pooled and presented thereafter as the response to linalool or to geraniol.

### 3.1. Spontaneous responses

The comparison of the frequency of spontaneous responses to geraniol and linalool revealed a significant difference ($\chi^2 = 27.2$, $P < 0.05$); more bees had spontaneous responses to linalool.

The data showed a significant effect of age on the frequency of spontaneous response to linalool and to geraniol (Fig. 1; $\chi^2 = 27.7$, $P < 0.01$ for linalool; $\chi^2 = 53.3$, $P < 0.001$ for geraniol). Younger bees exhibited the highest percentages of spontaneous proboscis extension: 35% of the 2-day-old bees responded to linalool, and 33% of the 4-day-old bees responded to geraniol. When stimulated with linalool, 15–25% of the 4- to 14-day-old bees showed spontaneous response, while older bees exhibited less than 10%. All bees from 6-day to 20-day-old groups showed less than 13% spontaneous response to geraniol.

### 3.2. Acquisition of the conditioned response

The level of response at the end of the conditioning period (pairing the odour with sucrose reward) did not differ with the odour stimulus ($\chi^2 = 2.6$, *NS*).

However, a significant effect of age was demonstrated for both odour stimuli (Fig. 2; $\chi^2 = 21.5$, $P < 0.05$ for linalool; $\chi^2 = 20.7$,

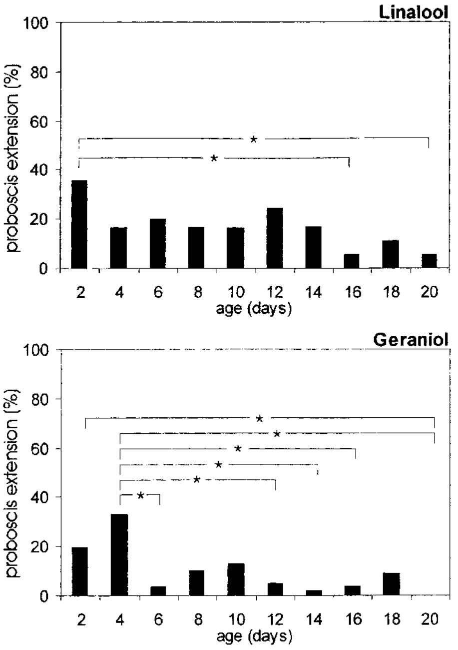

**Figure 1.** Percentages of bees exhibiting spontaneous proboscis extension to linalool and geraniol, presented as a function of their age. Sample sizes were comprised between 50 and 69 individuals. The statistics show a global heterogeneity of the responses according to age with both odours. Significant two-by-two comparisons are indicated (* $P < 0.0014$ according to the Dunn-Sidák adjustment for pairwise comparisons).

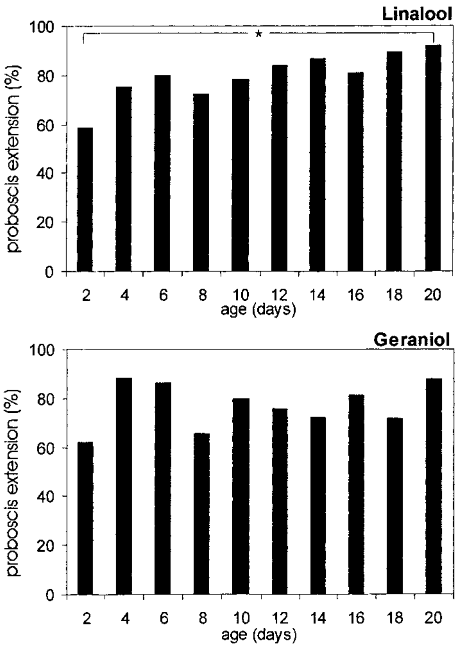

**Figure 2.** Percentages of bees exhibiting conditioned proboscis extension to linalool and geraniol at the end of the training period, presented as a function of their age. Sample sizes were comprised between 34 and 60 individuals. The statistics show a global heterogeneity of the responses according to age with both odours. Significant two-by-two comparisons are indicated (* $P < 0.0014$ according to the Dunn-Sidák adjustment for pairwise comparisons). Two-by-two comparisons did not indicate significant differences with geraniol.

$P < 0.05$ for geraniol). The frequency of conditioned responses to linalool increased progressively from 2-day to 20-day-old bees, with a significant difference between the two extreme age-groups. In the experiments with geraniol, conditioned responses were also the lowest in 2-day-old bees, but reached the highest values in 4-day and in 20-day-old bees with no linear variation among the intermediate ages. Pairwise comparisons of the conditioned responses to geraniol did not reveal significant difference between ages.

### 3.3. Resistance to extinction

The level of response at the end of the extinction period (presentation of the odour without subsequent reward) did not differ according to the odour stimulus ($\chi^2 = 15.6$, *NS*).

A highly significant age effect was demonstrated by the responses at the end of the extinction procedure (Fig. 3) for both linalool ($\chi^2 = 35.7$, $P < 0.001$) and geraniol ($\chi^2 = 24.6$, $P < 0.01$). For both odours, the

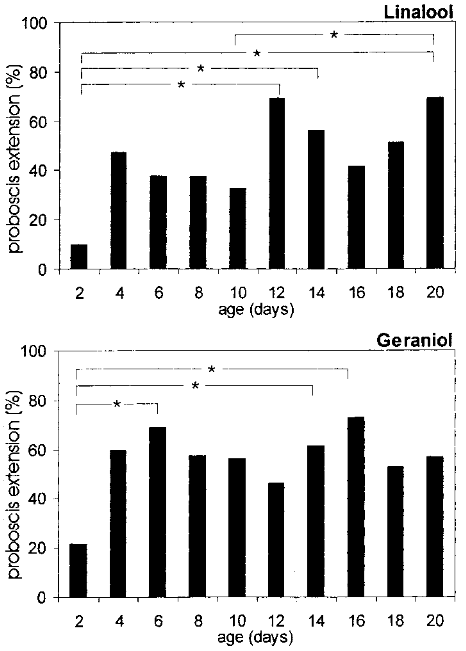

**Figure 3.** Percentages of bees exhibiting conditioned proboscis extension to linalool and geraniol at the end of the extinction period, presented as a function of their age. Sample sizes were comprised between 20 and 48 individuals. The statistics show a global heterogeneity of the responses according to age with both odours. Significant two-by-two comparisons are indicated (* $P < 0.0014$ according to the Dunn-Sidák adjustment for pairwise comparisons).

pairwise comparisons indicated that 2-day-old bees were less resistant to extinction since they exhibited the lowest percentage of response. With geraniol, the responses of other age-groups varied from 45 to 73%, with 6-, 14- and 16-day-old bees responding significantly more than the 2-day-old group. No significant difference was found among 4-day to 20-day-old bees. With linalool, the resistance to extinction appeared slightly weaker; the responses of 4- to 20-day age-groups varied from 32 to 70%, with 12- 14- and 20-day-old bees exhibiting significantly more responses than 2-day-old bees. Although no clear tendency could be drawn from the variations in the responses of 4- to 20-day-old bees, resistance to extinction with linalool seemed to be more accurate in bees older than 10 days.

## 4. DISCUSSION

The experiments have shown that the conditioned proboscis extension responses of worker bees were age-dependent. While previous works only explored the acquisition process, we demonstrated that two other parameters also varied with age, the frequency of spontaneous responses and the resistance to extinction. We obtained the weakest influence of age on the conditioned responses at the end of the acquisition period, but highly significant variations with age were observed for spontaneous responses and resistance to extinction.

When considering the three parameters together, proboscis extension responses of the youngest bees (less than 4 days of age) generally differed from those of older individuals. Young bees showed more spontaneous responses than older ones. The highest levels of spontaneous proboscis extension were obtained with 2-day-old bees when linalool was tested, and both 2-day and 4-day-old bees with geraniol. In addition, young bees showed poor acquisition performance. With linalool, 2-day-old bees exhibited only 59% conditioned responses, this level tending to increase with age. This trend was not completely reproduced with geraniol, however 2-day-old bees also showed the lowest level of conditioned responses with 62% bees exhibiting proboscis extension to the odour. Finally, during the extinction period, 2-day-old bees showed the lowest resistance to repeated unrewarded presentations of the conditioned stimulus.

We hypothesise that the performance exhibited by the youngest bees may have a neuro-developmental basis since the olfactory system is still maturing during the first days of the adult life. Functional morphology and neural studies have shown that the olfactory system is highly flexible in response to environmental changes until at least 4 days after emergence, its maturation being achieved between 4 to 8 days of age (Masson and Arnold, 1987; Masson et al., 1993). High levels of spontaneous responses during the first days of adult life might be related to a higher sensitivity to the odours at this critical period, when the sensory environment could affect the setting up of the olfactory system. Poor learning performance of young bees (poor acquisition as well as poor resistance to extinction) also might be a direct consequence of the immaturity of olfactory nervous system.

The level of spontaneous proboscis extension was found to vary from zero to 35%, which is in agreement with the values reported in the literature (Bhagavan et al., 1994; Sandoz et al., 1995). Changes in the level of spontaneous responses with age were highly significant with both odour stimuli, responses to linalool being slightly higher than those obtained with geraniol. The difference between the spontaneous responses to linalool and to geraniol might be due to prior exposure to geraniol as this volatile is a component of the Nasonov pheromone produced by the workers. One could hypothesise that pre-exposure to this compound could have induced sensory adaptation of the olfactory system, so that with subsequent exposure, the odour becomes less easily detected by the bees. In this case, it is not surprising to observe a decrease in spontaneous responses to geraniol as the bees aged, i.e. as the exposure continued. Moreover, Nasonov pheromone is produced in amounts that increase with workers' age (Boch and Shearer, 1963), and the effect of pre-exposure might be stronger in parallel. In this regard, our data do not support the conclusions of Ray and Ferneyhough (1997) who did not find any variation in the degree of spontaneous proboscis extension to geraniol between the different age-groups they tested. For floral compounds, Bhagavan et al. (1994) did not find any significant variation in the spontaneous responses to hexanal and 1-hexanol. However, in one of their experiments these authors observed a tendency for a decrease in spontaneous responses from young to old bees which is consistent with our results with linalool.

The effect of age on the acquisition process was found to be very weak. Except for 2-day-old bees, which exhibited a slightly lower level of conditioned responses, all bees were able to learn both linalool and geraniol efficiently. These results are consistent with the data reported by Ray and Ferneyhough (1997) who did not find changes in the levels of olfactory conditioning after 5 days of age. However they differ from previous results by Pham-Delègue et al. (1990) who showed a significant variation in the probability of responses between different age-groups from 2-day to 20-day-old bees. This discrepancy might be due to the conditioning procedure as Pham-Delègue et al. subjected the bees to five conditioning trials while we used three trials. The number of conditioning trials could affect both the degree of satiation and exhaustion in the bees, which could be a source of variability in the learning performances. Moreover, other factors are known to influence learning performance such as the season (Erber, 1981), race (Abramson et al., 1997), or prior experience (Jakobsen et al., 1995). Also Ray and Ferneyhough (1997, 1999), by comparing young bees which could fulfill nursing activities to both young workers forced to forage precociously and to old foragers, suggested that the activities of the bees in the colony influenced the development of learning performance. Such factors could interfere with the analysis of age-related variations and explain the disparate conclusions of the previous works.

The extinction process has been often considered in the study of learning and memory in the honey bee (Menzel et al., 1974; Couvillon and Bitterman, 1980; Abramson and Bitterman, 1986; Sandoz et al., 1995), but it has never been related to the age of bees. Our results showed a highly significant effect of age on the resistance to extinction. As we discarded the individuals which did not respond at the end of the previous acquisition period, we could assume that the differences between age groups were not related to a difference in their learning performance (i.e. all groups displayed equivalent conditioning levels, and all individuals were subjected to the same number of trials). The age-effect observed was mainly due to the lowest resistance of 2-day-old bees, as already discussed, but also to some variations in the responses of older bees. The variation in resistance to extinction observed in our study follow the age effect obtained in the acquisition phase by Pham-Delègue et al. (1990). Such variation may rely on the physiological state of the bees; i.e. the motivation for food, or the resistance to exhaustion when the experimental procedure lasts for many trials. Resistance to extinction may also be interpreted as a confirmation of behavioural rigidity which develops with ageing. Our results are consistent with reports of more conservative tendencies in older individuals, especially in the fruit fly (Fresquet, 1999).

In addition, in the honey bee it may be hypothesised that the variation in learning performance according to age should be related to the tasks the bee has to perform. Indeed, differential responses to odours may account for the performing of tasks implying the use of olfactory signals. Our data, particularly with linalool, revealed that bees over 12 days had a higher resistance to extinction compared to younger ones. Worker bees start foraging at this age on average under natural conditions (Michener, 1974; Seeley, 1982; Kolmes, 1985). Thus this consistency in their response may be related to the need to memorise and retain floral odours over successive foraging trips.

Our work has tentatively investigated the influence of ageing on olfactory learning capacities. However, an age effect on the behaviour may rely on various factors such as maturational processes, prior olfactory experience, and physiological state. Combined neurophysiological and behavioural recordings, performed at different ages, could help assess neural correlates of behavioural development. The genetic component should be also considered in future works on the ontogeny of olfactory learning since subfamily differences in the age at which workers began foraging has been described (Kolmes et al., 1989; Robinson, 1992). Investigations on these different aspects are still needed to evaluate whether age-related variations in odour learning abilities could play a role in task specialisation and therefore the division of labour.

## ACKNOWLEDGEMENTS

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**Résumé – Variations des performances d’apprentissage olfactif en fonction de l’âge des ouvrières d’abeille.** L’apprentissage olfactif est impliqué dans diverses activités réalisées à des moments différents au cours de la vie des ouvrières. On peut supposer que les capacités d’apprentissage varient en fonction des tâches et, en conséquence, en fonction de l’âge. Bien que le conditionnement de l’extension du proboscis ait été largement utilisé dans les études sur l’apprentissage olfactif chez l’abeille, peu de travaux se sont intéressés à la variabilité de cette réponse en fonction de l’âge, leurs conclusions étant d’ailleurs controversées. Dans le travail présenté ici, nous avons analysé les variations des performances d’apprentissage, lors d’un conditionnement de l’extension du proboscis, en fonction de l’âge des ouvrières. Les abeilles (*Apis mellifera* L.) sont prélevées à l’émergence sur des cadres de couvain, et maintenues par lots d’âge identique dans des cagettes d'élevage. Nous avons testé dix groupes, âgés respectivement de 2, 4, 6, 8, 10, 12, 14, 16, 18 et 20 jours. Les ouvrières sont soumises à une procédure constituée de trois essais de conditionnement suivis de cinq essais d'extinction, le stimulus odorant étant soit du linalol soit du géraniol. Trois réponses sont enregistrées au cours de la procédure expérimentale : (1) la réponse spontanée à l'odeur avant le conditionnement, (2) la performance d'acquisition à la fin de la phase de conditionnement, et (3) la résistance de la réponse conditionnée à la fin de la phase d'extinction.

En premier lieu, les résultats révèlent un effet significatif de l'âge sur les réponses spontanées, tant avec le linalol qu'avec le géraniol (Fig. 1). Les abeilles les plus jeunes montrent le plus d'extensions spontanées du proboscis, 35 % des ouvrières âgées de 2 jours répondant au linalol, et 33 % de celles de 4 jours répondant au géraniol. Par ailleurs, nous avons observé un effet significatif de l'âge sur la performance d'acquisition (Fig. 2). Avec le linalol, le taux de réponse augmente lentement entre les abeilles âgées de 2 jours et celles de 20 jours. Avec le géraniol, les ouvrières de 2 jours présentent la plus faible performance d'acquisition, mais des taux de réponse élevés sont observés dès l'âge de 4 jours. Enfin, les résultats indiquent aussi un effet significatif de l'âge sur la résistance à l'extinction (Fig. 3), les abeilles de 2 jours montrant une moindre résistance que les individus plus âgés. Chez ces derniers, les taux de réponse obtenus avec le géraniol varient de 45 à 73 % sans différence significative entre les groupes. Avec le linalol, la résistance à l'extinction semble un peu plus faible, les taux de réponse variant de 32 à 70 %. Bien qu'il ne soit pas possible de mettre en évidence une tendance nette, il semble que la résistance à l'extinction, avec le linalol, soit plus importante chez les abeilles âgées de plus de 10 jours.

Les réponses des ouvrières les plus jeunes (âgées de 2 et 4 jours) diffèrent de celles des individus plus âgés pour l'ensemble des trois paramètres étudiés, ce qui pourrait être dû au développement du système olfactif. Au cours des premiers jours de la vie imaginale, le système olfactif poursuit sa maturation et est particulièrement plastique en réponse aux changements de l'environnement. Les niveaux élevés de réponses spontanées pourraient être liés à une sensibilité supérieure à l'environnement olfactif au cours de cette période critique, alors que les faibles performances d'acquisition et de résistance à l'extinction, au même âge, pourraient être une conséquence directe de l'immaturité du système. À partir de 6 jours, seule la résistance à l'extinction varie significativement avec l'âge des ouvrières. Les variations des capacités d'apprentissage olfactif, en fonction de l'âge des abeilles, pourraient jouer un rôle dans la spécialisation comportementale des individus et, en conséquence, dans la division du travail au sein de la colonie.

**abeille / *Apis mellifera* / extension conditionnée du proboscis / effet âge**

**Zusammenfassung – Altersabhängigkeit in der Leistung bei olfaktorischer Konditionierung von Arbeitsbienen (*Apis mellifera* L.).** Das Erlernen von Düften steht mit den unterschiedlichen Aufgaben in den verschiedenen Lebensabschnitten der Arbeitsbienen im Zusammenhang. Wir untersuchten die Hypothese, dass die Lernfähigkeit sich mit den Aufgaben und dementsprechend mit dem Alter ändert. Der konditionierte Rüsselstreckreflex wird allgemein in Untersuchungen über das olfaktorische Lernen bei Honigbienen angewendet. In dieser Arbeit wollten wir der Frage über die Altersabhängigkeit der Rüsselstreckreaktion nachgehen. Bienen der italienischen Rasse *A. m. ligustica* wurden bei Schlupf von den Waben gesammelt und in Gruppen gleicher Altersstufen gekäfigt. Wir testeten Gruppen im Alter von 2, 4, 6, 8, 10, 12, 14, 16, 18 und 20 Tagen. Es wurden jeweils 3 Konditionierungsversuche durchgeführt,

denen 5 Extinktionsversuche angeschlossen wurden, und zwar entweder mit Linalool oder Geraniol als Duftreiz. Drei Parameter wurden während des Versuchs gewertet: 1. Grad der spontanen Antwort vor der Konditionierung, 2. Grad der Erlernung am Ende der Konditionierungsphase, und 3. Erhalt der Konditionierung am Ende der Extinktionsphase.

Als erstes Ergebnis zeigte sich ein signifikanter Alterseffekt bei der spontanen Rüsselstreckreaktion sowohl bei Linalool und Geraniol (Abb. 1). Bei den jüngsten Bienen erfolgte die stärkste Reaktion mit 35 % bei Linalool bei 2 Tage alten und 33 % Reaktion auf Geraniol bei 4 Tage alten Bienen. Ausserdem fanden wir einen signifikanten Einfluss des Alters auf den Grad der Erlernung (Abb. 2). Bei Linalool nahm die Anzahl der Reaktionen von 2 Tage alten Bienen langsam bis zum Alter von 20 Tagen zu. Bei Geraniol war das Lernen bei 2 Tage alten Bienen am schlechtesten, aber bei 4 Tage alten ergaben sich bereits hohe Lernwerte. Als drittes fanden wir einen signifikanten Alterseffekt beim Erhalt der Konditionierung (Abb. 3), wobei bei 2 Tage alten Bienen die Konditionierung signifikant schneller erlöscht als bei älteren. Nach einem Alter von 4 Tagen variierte die Reaktion zwischen 45 und 73 % ohne Unterschied zwischen den Gruppen. Die Extinktion bei Linalool erschien leichter zu erfolgen, die Reaktion von 4 bis 20 Tage alten Altersgruppen variierte zwischen 32 und 70 %. Auch wenn es keine eindeutige Tendenz in der Variation der Reaktionen dieser Gruppen gibt, scheint die Verhinderung der Extinktion bei Linalool vor allem bei Bienen älter als 10 Tage deutlich ausgeprägt zu sein.

Die Leistung der jüngsten Bienen (2 und 4 Tage alt) unterschied sich bei allen Parametern von denen der älteren. Das könnte mit der Ontogenie des olfaktorischen Systems zusammenhängen. In den ersten Tagen des adulten Lebens reift das olfaktorische System noch und ist in seinen Reaktionen auf Umweltänderungen noch sehr flexibel. Der hohe Grad an Spontanreaktionen in diesem Alter mag mit einer besonderen Empfindlichkeit auf die olfaktorische Umwelt in dieser kritischen Zeitspanne zusammenhängen. Dagegen könnte das geringe Lernvermögen und das geringe Widerstandsvermögen gegen die Extinktion im gleichen Alter eine direkte Folge des noch nicht voll entwickelten olfaktorischen Nervensystems sein. Erst bei älteren Bienen (ab dem Alter von 6 Tagen) wurde gefunden, dass das Widerstandsvermögen gegen die Extinktion sich mit dem Alter ändert. Diese altersabhänigige Variation in der Fähigkeit, Düfte zu erlernen, sollte eine Rolle in der Spezialisierung im Verhalten der Arbeiterinnen und damit in der Arbeitsteilung des Volkes spielen.

**Honigbiene / *Apis mellifera* / konditionierter Rüsselstreckreflex / Alter**

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