Ontogeny of colonial hydrocarbon label in callow workers of the ant *Cataglyphis iberica*

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**Abstract** - In ants, postpharyngeal glands are the reservoir for the colonial odour which mediates the interindividual recognition. Quantitative and qualitative changes in colonial hydrocarbon profile of these glands were studied in the ant *Cataglyphis iberica* from emergence of workers. Isolation of callow seems to affect the maturation process. The glandular secretion of the callow workers increases in amount and becomes similar to that of mature workers around 10 d old. However, the rate of hydrocarbon accumulation in the glands of callow workers that were reared in isolation remains lower compared to mature nestmates. Early social isolation also affects the acquisition of the specific colony profile which remains very different from that of their mother colony. These results suggest a transfer of hydrocarbons from matures to callows. This transfer allows the new members of the colony to integrate the colonial odour during the few days following emergence. (© Académie des sciences / Elsevier, Paris.)

postpharyngeal glands / ontogeny / colonial odour / social isolation / Cataglyphis / ant

**Résumé** - Chez les fourmis, les glandes postpharyngiennes constituent le réservoir de l’odeur coloniale qui permet la reconnaissance entre individus. L’évolution quantitative et qualitative du profil colonial d’hydrocarbures de ces glandes a été étudiée chez la fourmi *Cataglyphis iberica*, dès l’émergence des ouvrières. L’isolement social des ouvrières nouveau-nées semble affecter le processus de maturation de ce profil. Le contenu glandulaire des jeunes ouvrières augmente pour rejoindre celui des ouvrières matures vers l’âge de 10 j. Cependant, les ouvrières de même âge, isolées depuis leur éclosion imaginaire, gardent un contenu plus faible comparé à celui de leurs congénères matures. L’isolement précoce perturbe également l’acquisition du profil d’hydrocarbures qui demeure très différent de celui de la colonie mère. Ces résultats suggèrent un transfert des hydrocarbures des matures vers les ouvrières jeunes. Ce transfert permet aux nouveaux membres de la colonie d’intégrer l’odeur coloniale durant les jours qui suivent l’éclosion. (© Académie des sciences / Elsevier, Paris.)

glandes postpharyngiennes / ontogenèse / odeur coloniale / isolement social / Cataglyphis / fourmi
Version abrégée

La fermeture coloniale est une caractéristique largement répandue chez les sociétés de fourmis. Cette fermeture est liée à la présence d’un signal de reconnaissance de nature chimique (appelé improprement « odeur spécifique » bien qu’il s’agisse de substances très peu volatiles) qui permet la discrimination de tout intrus étranger à la colonie. Les nombreux études portant sur ce phénomène concordent sur l’implication des hydrocarbures cuticulaires dans la composition de l’odeur coloniale. Par ailleurs, les glandes postpharyngiennes (GPP), présentes uniquement chez les fourmis, constituent le lieu de stockage des hydrocarbures cuticulaires formant ainsi le réservoir de l’odeur coloniale. Cependant, à une reconnaissance coloniale rigide s’oppose une tolérance intercoloniale à l’égard des jeunes individus. Ce paradoxe nous a conduits à l’étude des modalités de mise en place de l’odeur coloniale chez les jeunes ouvrières. Nous avons choisi l’espèce Cataglyphis ibex dont le contenu des GPP est déjà connu.

Nous avons suivi l’évolution quantitative du contenu des GPP chez des jeunes ouvrières depuis l’élosion imaginale jusqu’à l’âge de 10 j (0, 1, 4, 7 et 10 j). Nous avons abordé également le rôle de la présence des congeneres matures dans l’ontogénèse du profil d’hydrocarbures de ces glandes. Les résultats sont comparés à ceux obtenus chez des ouvrières matures (> 3 mois d’âge) possédant déjà l’odeur coloniale caractéristique.

Chez les ouvrières maintenues au sein de leur colonie mère après l’élosion (témoins), le contenu glandulaire, très faible à l’émergence, s’accroît progressivement durant les 10 premiers j pour atteindre un taux moyen (- 840 ± 120 ng) comparable à celui observé chez leurs congénères matures dont le contenu est très variable (- 1260 ± 420 ng). Les ouvrières de même âge, isolées dès l’élosion, montrent également une évolution croissante de leur contenu glandulaire mais dont le poids reste significativement faible au bout de dix jours (- 530 ± 160 ng), comparé à celui de leurs congénères matures (- 1560 ± 360 ng). Le poids corporel des ouvrières émergentes ne semble pas interférer avec cette évolution puisque celui-ci ne change pas significativement avec l’âge ni selon les colonies.

L’analyse du profil d’hydrocarbures des jeunes immatures, indépendamment de l’effet de l’isolement, met en évidence une odeur bien distincte de celle de leurs congénères matures. De plus, chaque classe d’âge étudiée possède un profil caractéristique différent de celui des autres classes d’âge. Cependant, dans des conditions normales où les jeunes sont maintenues au sein de la colonie mère après l’élosion, le profil évolue graduellement avec l’âge en convergent vers celui des matures, si bien qu’à l’âge de 7-10 j ce profil devient très proche de celui des matures. Les ouvrières isolées présentent également ce caractère dynamique du profil malgré l’isolement social précoce, mais la convergence de celui-ci vers le profil des témoins matures disparaît, si bien qu’à l’âge de 7-10 j le profil des ouvrières demeure nettement distinct de celui des matures. L’acquisition du profil colonial caractéristique n’est donc pas accomplie avant ou à l’émergence des ouvrières, mais intervient au bout d’une dizaine de jours après au sein de la colonie mère, durant lesquels les ouvrières jeunes ajustent leur odeur à celle de la colonie. Ces résultats suggèrent un transfert préférentiel d’hydrocarbures des ouvrières matures vers leurs congénères jeunes.

1. Introduction

Nestmate recognition is one of the major points of interest in social biology. Many studies have led to the assumption that in social insects members of a colony have a common odour (1-2). Cuticular substances, mainly composed of hydrocarbons, seem to be involved in nestmate recognition, and experiments with chemicaly treated surrogates support this [3]. More recent experiments conducted on the ant Cataglyphis niger, which analysed the behavioural modifications induced by isolated hydrocarbon fraction, confirmed that role (Lahav et al., in prep.). The postpharyngeal gland (PPG), which occurs only in Formicidae, contains hydrocarbons similar to those found on the cuticular surface [4-6]. Using radio-labelled hydrocarbons, it was shown that the PPG stores hydrocarbons after their synthesis in the cuticular epithelium and transportation by the haemolymph, hydrocarbons are then reapplied on the cuticle through selfgrooming. The PPG is also involved in the distribution of hydrocarbons between nestmates through trophallaxis and allogrooming, serving therefore as a ‘Gestalt organ’ [7-9]. These data were confirmed on Camponotus vagus by topical applications of (Z)-9-tricosene which is not a natural hydrocarbon in this species [10].

On the intracolonial scale, recognition appears at different levels. This phenomenon has been behaviourally demonstrated in Camponotus floridanus with artificial colonies including several distinctive matrilines [11]. In bees, recent data have shown that workers of different matrilines have hydrocarbon profiles that differ significantly, which could be related to preferential trophallaxis directed towards full nestmates [12]. This discrimination originates from chemical cues characteristic of the individual identity, which is maintained even though the chemical cues are partially mixed, constituting the colonial identity. The discrimination tied to workers’ age and their social status among the same colony remains relatively unknown. Camponotus vagus is the only species where an intracolonial discrimination based on the social status of workers was observed. The foragers (the oldest workers) of this species are able to recognize homocolonial brood-tenders (young workers) and to carry them into the nest [13]. These authors hypothesized that cuti-

cicular hydrocarbons serve as a cue that allows foragers to discriminate between members of the two castes.

Previous studies on the specific behaviour of young workers have greatly contributed to our understanding of their integration process into the mother colony [114]. In contrast, a physiological approach following the chemical changes and the acquisition of the label has rarely been investigated. The ontogeny of the PPG has been studied only for structural and chemical aspects in Cataglyphis niger [6]. In the present work, we studied ontogenetic changes in the PPG profile quantitatively and qualitatively in order to assess the existence of a chemical profile characteristic of callow workers and to understand the process of colony label acquisition in young Cataglyphis iberica workers. The constituents of the PPG content of C. iberica were previously identified [115].

2. Material and methods

Cataglyphis iberica is a monogynous and polydomous species that lives in the semi-arid habitats of the Iberian Peninsula [16]. Each colony is made up of several satellite nests (in one of them resides the single queen) connected by the exchange of adult workers through social carrying [117]. Age polyethism related to adult transport was studied behaviourally [118].

In this study, we used workers of two different colonies, α and β, collected in August 1995 in Barcelona (Spain). Each colony was composed originally of three nests and contained several hundred workers and brood. After being collected the nests of each colony were joined into a single nest. The colonies were reared in the laboratory in a plastic box (11.5 x 8.0 x 2.5 cm) connected to a foraging area (35 x 35 cm) and placed under the following conditions: 50 % humidity; temperature: 25 ± 2 °C; photoperiod 10:14 D/N. Workers of α colony were labelled with a dot of paint upon emergence according to age groups and returned to their colony and dissected to examine the content of their PPG. In the β colony, callow workers were removed at emergence and kept isolated individually until they were dissected. Mature workers of α and β colonies were used as controls. The emergence of callow workers was verified at least twice a day.

We studied six age groups: 0 d (workers removed from their cocoon just before emergence), 1, 4, 7 and 10 d and mature workers (workers older than 3 months taken from the foraging area). We conducted the chemical analysis according to earlier experiments [119, 120]. We used the PPG secretion to avoid contamination from other glandular sources, and acetone as solvent since the extracts are identical to those obtained with pentane in Cataglyphis ants [114]. The PPG of five workers from each age group for the two colonies except for mature workers in which n = 8) were dissected in distilled water. Each gland was then immersed individually in 100 μL acetone for 24 h in order to extract the gland content. Each extract was dried under nitrogen and diluted in 15 μL of acetone of which 2 μL were taken for analysis by gas-chromatography (DEL51 300 chromatograph with a capillary column Chrompack CPSIL 5 WCOT, length: 25 m, diameter: 0.22 mm, temperature programmed from 100 to 280 °C at 3 °C/min). Quantification was achieved by peak integration (ENICA 21 integrator using the following formula: W = TPS*(Wes/Ses)*7.5 where W is the calculated weight of the PPG secretion in ng; TPS is the total peak surface of the 2 μL sample; Wes is the weight of the external standard used (octadecane) in ng; Ses is the peak surface of the external standard; 7.5 is the dilution factor).

In order to evaluate the degree of similarity of the different age group profiles, we conducted a factorial analysis of correspondences for each colony on the basis of relative proportions of 29 major PPG hydrocarbons in young and mature individuals (figure 1). All 0-d-old workers and one 1-d-old worker from α colony were excluded from the analysis because their glandular contents were too low for quantitative analysis. The degree of homogeneity of the different groups was represented on the diagrams by the elliptic surface which corresponds to the standard deviations calculated with the co-ordinates of the cloud points on the two axes.

3. Results

Table 1 shows the mean total content of the PPG expressed in ng for each age group for the two colonies. The weight of the workers was 7-10 mg without differences between age groups and colonies (ANOVA, Newman-Keuls test, P > 0.05), enabling us to directly compare the glandular contents. The PPG content was quantitatively similar for mature workers of both colonies (1257 versus 1560 ng, no significant difference due to large interindividual variations). This content changed with worker age; but was never significantly different between the two colonies. In colony α, it increased from 6.9 % at emergence to 67 % of the mature weight at 10 d. It was significantly lower for young workers (0, 1, 4 and 7 d) than for mature homocolonial workers, being intermediate for 10 d. In the isolated β workers we globally observed the same modifications, but the glandular content of 10 d old workers was not different from that of young workers and was still much lower than that of mature ones (reaching only 31.8 % of the mature PPG amount).

Factorial analysis of correspondences performed on profiles revealed the great similarity of the mature spectra which constitutes in the two colonies a very homogeneous group. It also showed that the other age groups displayed very heterogeneous profiles but that they tend to possess their own specific labels. Furthermore, the profile of young workers changed gradually with age, ranging from the profile of a 1-d-old individual to that of a 10-d-old individual which is close to the mature profile, but
nevertheless different. The age groups of the β colony also changed gradually, while remaining very different from the mature controls of the mother colony (figure 2 α and β).

To evaluate whether young individuals have a specific colony profile in spite of their heterogeneity, we conducted an ascendant hierarchical analysis on the profiles of mature and 4-d-old individuals in both colonies.

Figure 1. Chromatograms of postpharyngeal gland content in a mature and a young (7-d-old) worker of Cataglyphis iberica, with the chemical identification of the 29 major peaks.

See the text for more details on the chromatographic characteristics (m and dm correspond to methyl and dimethyl, respectively).
Table I. Changes in the amount of the postpharyngeal gland content expressed in ng (mean ± SE) according to worker age of α and β colonies in Cataglyphis iberica.

<table>
<thead>
<tr>
<th></th>
<th>0-d-old</th>
<th>1-d-old</th>
<th>4-d-old</th>
<th>7-d-old</th>
<th>10-d-old</th>
<th>Matures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony α</td>
<td>87.4 ± 30.8 a</td>
<td>303.9 ± 162.9 a</td>
<td>284.8 ± 76.3 a</td>
<td>327.4 ± 79.8 a</td>
<td>842.7 ± 118.1 a, b</td>
<td>1257.0 ± 421.5 b</td>
</tr>
<tr>
<td></td>
<td>6.9 %</td>
<td>24.1 %</td>
<td>23 %</td>
<td>26 %</td>
<td>67 %</td>
<td>100 %</td>
</tr>
<tr>
<td>Colony β</td>
<td>60.1 ± 7.4 a</td>
<td>214.1 ± 55.6 a</td>
<td>200.2 ± 44.7 a</td>
<td>284.8 ± 62.5 a</td>
<td>527.3 ± 162.2 a</td>
<td>1560.3 ± 359.5 b</td>
</tr>
<tr>
<td></td>
<td>3.0 %</td>
<td>13.7 %</td>
<td>12.9 %</td>
<td>18.2 %</td>
<td>31.8 %</td>
<td>100 %</td>
</tr>
<tr>
<td>α versus β</td>
<td>Mann-Whitney</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

For both colonies, letters indicate values which differ significantly (ANOVA, Newman-Keuls test, P < 0.05). % indicates the relative quantities of the PPG secretion compared to those of mature nestmates.

Insects, the changes in hydrocarbons with age have been essentially studied by comparing the different stages of development. Several studies report significant changes in cuticular hydrocarbons between the larval and the adult stage [21–23] and between young and mature individuals [24]. In ants, the cuticular hydrocarbon pattern of adult workers shows temporal changes [3]. These temporal changes have also been shown in the PPG hydrocarbon pattern of C. niger workers [61]. Our results with C. iberica workers show that the PPG content is modified with age: its amount increases gradually and slowly. At the age of 10 d, it is near the mature content but still inferior. These temporal changes are very similar to those observed in C. niger where these quantitative modifications are correlated to a parallel evolution of the epithelial thickness of the glandular lining [61]. Fiedler's hypothesis of progressive odour in ants [25] suggested a substantial and continuous change in the individual odour with age. This hypothesis has been verified in Camponotus floridanus [26]. The lack of external chemical characteristics of newborn callows, recently qualified as a 'cuticular chemical insignificance' [27], seems to be responsible for intercolonial adoptions and could explain the 'acceptance period' observed in many ant species [28]. On the other hand, mature as well as young workers of both colonies display a colony-specific hydrocarbon profile as was shown in C. floridanus [26]. In C. iberica a more complex phenomenon appears: worker hydrocarbon profiles are distinctive according to age group and gradually converge from a 'callow profile' with specific hydrocarbons to a profile characteristic of mature workers. This callow profile changes rapidly, and the mature profile becomes dominant and principally colonial specific as was already suggested [29]. However, independently of the rest of the spectrum, the possible role of pentacosane and its derivatives in the discrimination of young workers by older nestmates needs to be investigated.

Our experiments show that in C. iberica, the acquisition of the colonial label by callows occurs during the first days of imaginal life. These data are congruent with the fact that members of the same colony exchange chemical substances involved in the formation of the colonial label. This odour transfer occurs through mutual licking and trophallaxis, leading to the mixing and the homogenization of the odour in the entire colony [7]. The 'cuticular chemical insignificance' is consequently followed by 'chemical integration' [27]. In accordance with recent results the profile of ants reared within queenless groups was not affected by the lack of the queen, because in C. iberica the queen does not seem to be involved in the formation of the colonial odour [30].

Although we tested only two colonies, some conclusions can be drawn from the results of the isolation experiments. Ants reared in isolation exhibit profiles that change gradually with age, but remain very heterogeneous and different from the mature profile of the mother colony. On the contrary, ants reared within the mother colony display more homogeneous profiles which converge toward the mature profile. However, this
convergence is not so obvious for isolated workers. The acquisition of the characteristic mature profile does not occur as callows emerge, but is achieved through cohabitation in the mother colony. These results confirm, from a chemical view-point, some other behavioural studies [26, 31]. In C. cursor, callow workers are easily adopted in alien colonies when they are not older than 4 d. At that age, they are vigorously attacked, showing that the first days of adult life may represent a crucial period for young individuals to develop the colonial label [32].

Workers isolated from their mother colony also keep a dynamic profile, but it remains different from the mother colony. In Camponotus the reintroduction of young workers into their natal colony, after isolation prior to or just after their emergence, elicits aggressive behaviour from resident sisters [33]. The isolation of callow Ectatomma tuberculatum workers from their mother colony induces an irreversible perturbation on their behavioural ontogenesis [34]. However, the modification of the early social environment due to isolation can induce physiological
changes in workers. These changes can influence the quantity of synthesized hydrocarbons and thus modify their chemical profile.

In accordance with recent knowledge on colonial odour (see reviews [3, 27]), we can hypothesize that colony label would be acquired from the following two sources:

- an endogenous source which provides the primary material of the label by an increasing hydrocarbon production; this production seems to be independent of the presence of mature nestmates;

- an exogenous source which acts concomitantly through social interactions (mainly trophallaxis) with mature nestmates; by this means, callows adjust their hydrocarbon profile to the quantitative properties of the colony.

The process of odour transfer may be very important for C. iberica colonies, because their organization (polydomous) implies a temporary physical separation of workers, especially during hibernation, and the necessity to maintain the contacts between the different nests by social carrying. In studying mutual transport in C. iberica and its relation with the PPG content, we observed differences between carrier and carried workers: social transport is maximum at the end of hibernation (April–May) and carried workers display the same characteristics as the callows. In fact, these workers are young and easily discriminated because of their low PPG content, their profile is more heterogeneous and slightly different from that of the carrier workers [19]. The situation seems therefore similar to the discrimination of young callows by mature nestmates. The combination between the low level of the PPG content and the nature of the profile, could be the basis for the discrimination of young callows. This combination could induce transport as well as increased trophallactic exchanges and licking by mature nestmates which permit a transmission of chemical cues.

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5. References


