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# Individual, Geographical and Experimental Variation of Cuticular Hydrocarbons of the Ant *Cataglyphis cursor* (Hymenoptera: Formicidae): Their Use in Nest and Subspecies Recognition

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**Key Word Index**—*Cataglyphis cursor*; Hymenoptera; Formicidae; cuticle; hydrocarbons; colonial; odor; populations; aggressive behavior.

**Abstract**—The cuticular hydrocarbons of *Cataglyphis cursor* (Fonsc.) adults have been identified and quantified. Comparison of the proportion of hydrocarbons in various locations shows a variation between populations from each side of the Rhône river and an isolated population in the mountains near Madrid. In the studied area (not including Italy and eastern places) at least three subspecies have been found. Ethological analyses show that colony recognition, as indicated by aggressive behavior and the possibilities of adoption in an alien society, is correlated with the composition of cuticular hydrocarbons. Callows can be adopted in an alien colony, they can live in two colonies where the adults do not tolerate each other. These adopted callows have a hydrocarbon pattern intermediate between the patterns of the mother colony and the adoptive colony.

## Introduction

Hydrocarbons are major components of insect cuticular lipids. They prevent dessication and are important in chemical communication, particularly in species and caste recognition [1].

Bioassays developed by Howard *et al.* [2,3] support the species recognition role of hydrocarbons in two sympatric species *Reticulitermes* from Mississippi: *R. virginicus* and *R. flavipes*. The hydrocarbon of various species of *Reticulitermes* from France and the U.S.A. were characterized by Clément *et al.* [4] and those of *Zootermopsis* by Haverty *et al.* [5]. The four American species of *Solenopsis* can be readily identified by their hydrocarbon pattern [6]. Some staphylinid beetles such as *Trichopsenius frosti* have the same cuticular hydrocarbons as those of their host termite *R. flavipes* and are able to biosynthesize them. These cuticular hydrocarbons probably serve as the primary mechanism by which *Trichopsenius* integrates itself into the termite colony [7]. Three other staphylinid beetles associated with *R. virginicus* possess the same cuticular hydrocarbons as their host [8]. Chemical mimicry is also found in the myrmecophilous beetle *Myrmecaphodius excavaticollis* which lives in *Solenopsis richteri* colonies [9]. Espelie and Hermann [10] also found recently a chemical convergence of hydrocarbons between a social wasp, an ant and the host plant. A number of studies thus indicate that hydrocarbons could be used by social insects as species recognition pheromones. Intraspecific variation can also be observed: in *Reticulitermes* Bagnères *et al.* [11] found seasonal, intercolonial and intercaste variation; Bonavita-Cougourdan *et al.* [12] found different patterns between workers and larvae of *Camponotus vagus*.

Cuticular hydrocarbons are also used in biosystematics for the identification of sibling species. In these complex groups, separation of the adults by morphometric or biochemical techniques is very difficult, but identification by relative abundance of hydrocarbons is possible with multivariate analysis. Such techniques have been used for *Diptera*, for example the *Anopheles gambiae* complex [13], the *Simulium dominosum* complex [14] and amongst *Drosophila* [15, 16].

The problem of colony odor remains con-

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troversial. Volatile secretions of various glands have been implicated in ants [17, 18] and in bees [19, 20]. It is now well established that alarm and trail pheromones are colony, and sometimes individually, specific (e.g [21, 22]). But, even in a situation where individuals do not emit volatile pheromones, they can easily recognize nestmates at a short distance. Howse [23] hypothesized that in this situation non-volatile hydrocarbons could be concerned. The first experimental data were presented by Bonavita-Cougourdan *et al.* [24, 25] who showed that intercolony aggression in *Camponotus vagus* is correlated with variation in hydrocarbon levels. There are also differences in hydrocarbon patterns between callow ants and foragers, which permit subcaste discrimination. By washing ants in a solvent and transferring the substance to a lure, these authors were able to release aggression when introduced into an alien colony. This was the first demonstration of the role of hydrocarbons in colonial recognition. Morel and Vander Meer [26] obtained the same results in the American ant *Camponotus floridanus*. They also showed that isolation of the newborn ant prevents a normal development of hydrocarbon pattern. This explained previous results of Morel [27], who observed that young *Camponotus* workers, isolated at emergence, were attacked when they were later reintroduced into their colony.

Brill *et al.* [28–30] studying *Solenopsis invicta*, showed that it is possible to differentiate colonies by their hydrocarbon patterns. However, in this species, colonial odor seems to be largely influenced by rearing conditions: it is therefore not possible to correlate intercolonial aggressiveness with hydrocarbons [31].

*Cataglyphis cursor* (Fonsc. [32]) is an ant species which lives in the Mediterranean region, favoring dry places with little vegetation. Colonies are monogynous and monocalic [33, 34]. In this species intercolonial aggressiveness has been observed in the laboratory, and is related to the geographical distance separating the colonies [35]. It has also been observed that adoptions never occurred when the colonies involved came from east or west of the Rhône, which apparently divides two different populations. For these reasons the cuticular hydrocarbon variations of *C. cursor* from various places in the South of France and Spain were quantified, and relations between colonial aggressiveness and hydrocarbon patterns were measured.

## Results

### *Cuticular hydrocarbons*

The chromatograms reveal the existence of numerous peaks. More than 40 substances present in significant quantity (higher than 0.1%) could be identified. They are all hydrocarbons from C-25 to C-33 (Table 1): *n* alkanes, *n*C-25-*n*C-32; monomethylalkanes, C-25–C-32, dimethylalkanes, C-25–C-29; alkenes, C-25:1 (peak 1), C-27:1 (peak 11), C-31:1 (peak 34), C-32:1 (peak 37) and C-33:1 (peak 40).

### *Geographical variation of hydrocarbons*

Figure 1 shows chromatograms from two widely separated locations: Banyuls (Pyrénées-Orientales: 1A) and Apt (Provence: 1B). In Table 2 we report the composition of these two populations as well as the data for Madrid. Nearly all the quantities are significantly different as indicated by the *U*-test. Ants from Banyuls have more light hydrocarbons and the ants of Provence have the heaviest ones (the Nei index between the two groups is low: 0.4). Ants from Madrid are closer to Apt than to Banyuls, which is nearer geographically. A correspondence analysis (a form of principal component analysis [44]) was performed on the mean quantities (%) for each colony. Figure 2 shows a projection of the colonies on the two first axes. Factor one explains 53% of the variance and easily discriminates two groups: the first is situated to the east of the Rhône (Provence) and the second to the west of this river, up to Barcelona. The ants of Madrid are closely related to the Provence group. The second factor (13.9% of the variance) and the third factor (9.7% of the variance) revealed by the correspondence analysis enable us to distinguish two groups in the "Provence" population; one on the right edge of the Rhône and a second in the Madrid region, (Fig. 3). These groups are exactly superposed on the ethological isolation observed by Nowbahari [45]: the adoption of ants belonging to a different population by a foreign colony is completely impossible.

A hierarchical cluster analysis (ascending

TABLE 1. CUTICULAR HYDROCARBONS OF *CATAGLYPHIS CURSOR* ANTS IDENTIFICATION

| No. peaks | Carbon no. | Identification | $M_r$ | Diagnostic EI ions | $[M-H]^+$ | Diagnostic $CH_4$/CI ions |
|---|---|---|---|---|---|---|
| Alkanes | | | | | | |
| 2 | 25 | $nC_{25}$ | 352 | | 351 | |
| 7 | 26 | $nC_{26}$ | 366 | | 365 | |
| 13 | 27 | $nC_{27}$ | 380 | | 379 | |
| 17 | 28 | $nC_{28}$ | 394 | | 393 | |
| 25 | 29 | $nC_{29}$ | 408 | | 407 | |
| 31 | 30 | $nC_{30}$ | 422 | | 421 | |
| 38 | 32 | $nC_{32}$ | 450 | | 449 | |
| Monomethylalkanes | | | | | | |
| 3 | 26 | 11-Me $C_{25}$ | 366 | 168, 169, 224, 225 | 365 | 169, 225, 351 |
| | 26 | 13 Me $C_{25}$ | 366 | 196, 197 | 365 | 197, 351 |
| 4 | 26 | 5 Me $C_{25}$ | 366 | 84, 308, 309 | 365 | 309, 351 |
| 5 | 26 | 3 Me $C_{25}$ | 366 | 56, 336, 337 | 365 | 337, 351 |
| 9 | 27 | 10 Me $C_{26}$ | 380 | 154, 155, 252, 253 | 379 | 155, 253, 365 |
| | 27 | 11 Me $C_{26}$ | 380 | 168, 169, 238, 239 | 379 | 169, 239, 365 |
| | 27 | 13 Me $C_6$ | 380 | 196, 197, 210, 211 | 379 | 197, 211, 365 |
| 10 | 27 | 4 Me $C_{26}$ | 380 | 70, 336, 337 | 379 | 337, 365 |
| 14 | 28 | 11 Me $C_{27}$ | 394 | 168, 169, 252, 253 | 393 | 169, 253, 379 |
| | 28 | 13 Me $C_{27}$ | 394 | 196, 197, 224, 225 | 393 | 197, 225, 379 |
| 15 | 28 | 5 Me $C_{27}$ | 394 | 84, 336, 337 | 393 | 337, 379 |
| 16 | 28 | 3 Me $C_{27}$ | 394 | 56, 364, 365 | 393 | 365, 379 |
| 19 | 29 | *x* Me $C_{28}$ | 408 | | 407 | 393 |
| 26 | 30 | 11-Me $C_{29}$ | 422 | 168, 169, 280, 281 | 421 | 169, 281, 407 |
| | 30 | 13-Me $C_{29}$ | 422 | 196, 197, 252, 253 | 421 | 197, 253, 407 |
| | 30 | 15-Me $C_{29}$ | 422 | 224, 225 | 421 | 225, 407 |
| 27 | 30 | 5-Me $C_{29}$ | 422 | 84, 364, 365 | 421 | 365, 407 |
| 28 | 30 | 4-Me $C_{29}$ | 422 | 70, 378, 379 | 421 | 379, 407 |
| 32 | 31 | 13-Me $C_{30}$ | 436 | 196, 197, 266, 267 | 435 | 197, 267, 421 |
| | 31 | 15-Me $C_{30}$ | 436 | 224, 225, 238, 239 | 435 | 225, 259, 421 |
| 36 | 32 | 13-Me $C_{31}$ | 450 | 196, 197, 280, 281 | 449 | 197, 281, 435 |
| | 32 | 15-Me $C_{31}$ | 450 | 224, 225, 252, 253 | 449 | 225, 253, 435 |
| 39 | 33 | *x*-Me $C_{32}$ | 464 | | 463 | 435 |
| Dimethylalkanes | | | | | | |
| 6 | 27 | 5,9-diMe $C_{25}$ | 380 | 84, 155, 252, 323 | 379 | 155, 253, 323, 365 |
| 8 | 27 | 3,18-diMe $C_{25}$ | 380 | 56, 126, 281, 351 | 379 | 127, 281, 351, 365 |
| 12 | 28 | 4,8-diME $C_{26}$ | 394 | 70, 141, 280, 351 | 393 | 141, 281, 351, 379 |
| | 28 | 4,12-diMe $C_{26}$ | 394 | 70, 197, 224, 351 | 393 | 197, 225, 351, 379 |
| 18 | 29 | 3,11-diMe $C_{27}$ | 408 | 56, 183, 252, 379 | 407 | 183, 255, 379, 393 |
| 20 | 30 | 3,8-diMe $C_{28}$ | 422 | 56, 141, 308, 393 | 421 | 141, 309, 393, 407 |
| | | or 3,13-diMe $C_{28}$ | 422 | 56, 211, 238, 393 | 421 | 211, 238, 393, 407 |
| 23 | 30 | 6,10-diMe $C_{28}$ | 422 | 98, 169, 280, 351 | 421 | 169, 281, 351, 407 |
| | 30 | 6,18-diMe $C_{28}$ | 422 | 98, 168, 281, 351 | 421 | 169, 281, 351, 407 |
| 24 | 30 | 4,12-diMe $C_{28}$ | 422 | 70, 197, 252, 379 | 421 | 197, 253, 379, 407 |
| | 30 | 4,14-diMe $C_{28}$ | 422 | 70, 224, 225, 379 | 421 | 225, 379, 407 |
| 29 | 31 | 5,13-diMe $C_{29}$ | 436 | 84, 211, 252, 379 | 435 | 211, 253, 379, 421 |
| 30 | 31 | 3,11-diMe $C_{29}$ | 436 | 56, 183, 280,407 | 435 | 183, 281, 407, 421 |
| Alkenes | | | | | | |
| 1 | 25 | 9C25:1 | 350 | 173, 271, 444* | 351† | |
| 11 | 27 | 9C27:1 | 378 | 173, 299, 472* | 379† | |
| 34 | 31 | 8C31:1 + 9C31:1 + 10C31:1 | 448 | 159, 173, 187, 341, 355, 369, 528* | 449† | |
| 37 | 32 | 8C32:1 + 10C32:1 | 462 | 159, 187, 355, 383, 542* | 463† | |
| 40 | 33 | 9C33:1 | 476 | 187, 369, 556* | 477† | |

*After alkylthiolation.

†$MH^+$.

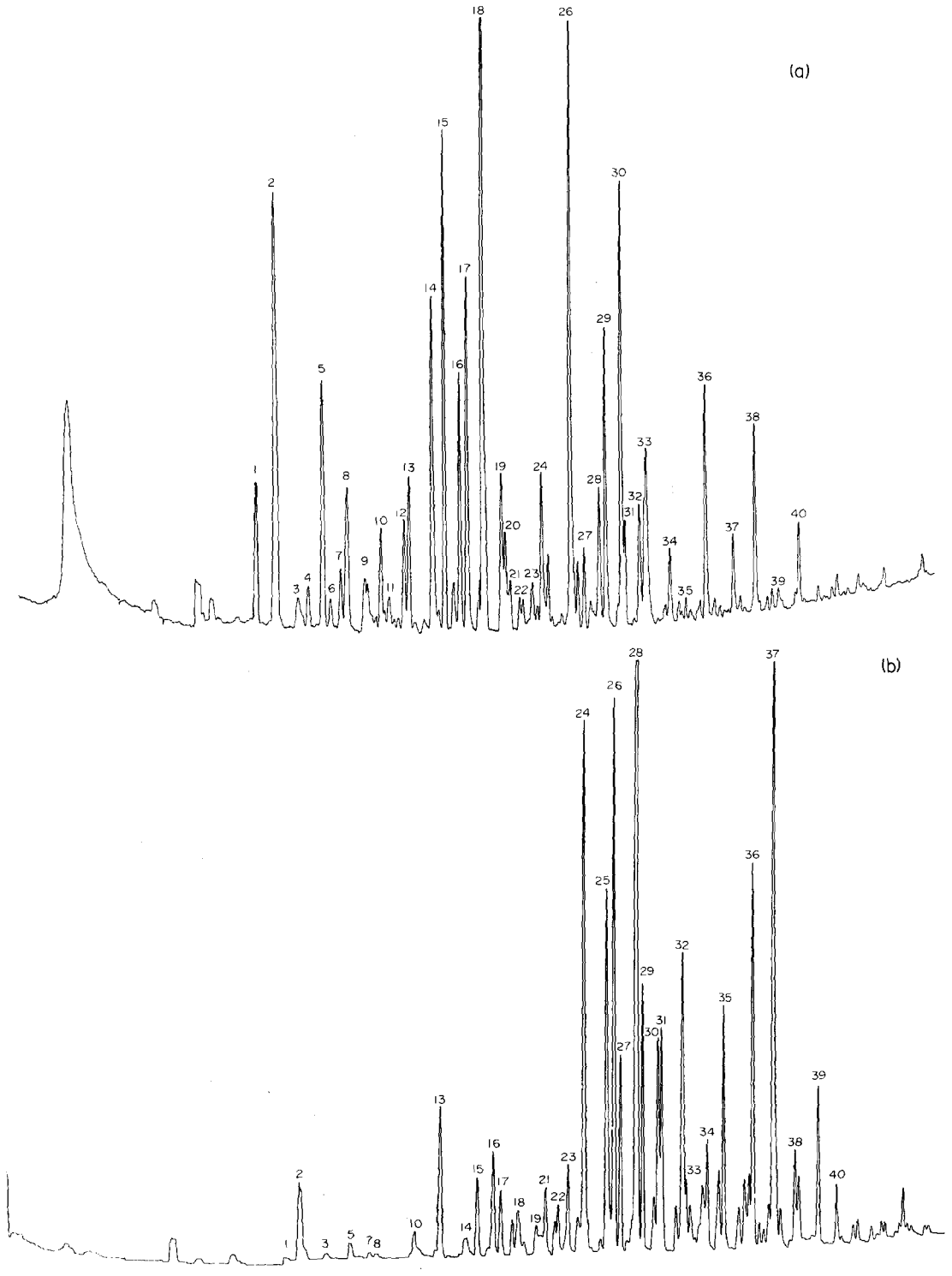


*FIG. 1.* GAS CHROMATOGRAMS OF CUTICULAR HYDROCARBONS OF ANTS *CATAGLYPHIS CURSOR* (WCOT apolar column CP-Sil-5, 25 *m*, 200–320°C running in 3°C/min). A, ant from Banyuls; B, *ant from Apt.*

TABLE 2. PROPORTIONS OF THE MAJOR CUTICULAR HYDROCARBONS OF *C. CURSOR* GROUP IN *C. CURSOR PILISCAPA* (= *TIBIALIS*) (Banyuls), *C. CURSOR CURSOR* (Apt) AND *C. CURSOR "MADRID"*

| No. name | *C. c. pili.* % $n=57$ | S.D. | *C. c. cur.* % n =46 | S.D. | *C. c. madri.* % $n=10$ | S.D. |
|---|---|---|---|---|---|---|
| 1. 9-$C_{25}$:1 | 0.798 | 0.555 | *t* | | *t* | |
| 2. *n*$C_{25}$ | 5.428 | 0.298 | 2.347 | 0.298 | 0.638 | 0.081 |
| 3. 11Me$C_{25}$+13Me$C_{25}$ | 0.432 | 0.211 | 0.271 | 0.28 | *t* | |
| 4. 5Me$C_{25}$ | 0.441 | 0.311 | *t* | | *t* | |
| 5. 3Me$C_{25}$ | 2.473 | 0.118 | 0.244 | 0.039 | *t* | |
| 6. 5,9diMe$C_{25}$ | 0.287 | 0.151 | *t* | | *t* | |
| 7. *n*$C_{26}$ | 0.641 | 0.318 | 0.249 | 0.415 | 0.189 | 0.116 |
| 8. 3,18diMe$C_{25}$ | 1.180 | 0.040 | 0.165 | 0.217 | *t* | |
| 9. 10Me$C_{26}$+11Me$C_{26}$+13Me$C_{26}$ | 0.640 | 0.330 | *t* | | *t* | |
| 10. 4Me$C_{26}$ | 1.153 | 0.050 | 0.204 | 0.037 | 0.100 | 0.054 |
| 11. 9-$C_{27}$:1 | 1.735 | 0.126 | 0.102 | 0.018 | 0.103 | 0.089 |
| 12. 4,8diMe$C_{26}$+4,12diMe$C_{26}$ | 1.201 | 0.053 | 0.169 | 0.022 | 0.129 | 0.077 |
| 13. *n*$C_{27}$ | 4.153 | 0.251 | 1.928 | 0.183 | 2.837 | 0.298 |
| 14. 11Me$C_{27}$+13Me$C_{27}$ | 2.364 | 0.095 | 0.711 | 0.062 | 3.315 | 0.566 |
| 15. 5Me$C_{27}$ | 4.713 | 0.197 | 0.913 | 0.040 | 0.489 | 0.070 |
| 16. 3Me$C_{27}$ | 2.777 | 0.146 | 1.113 | 0.050 | 0.932 | 0.038 |
| 17. *n*$C_{28}$ | 4.084 | 0.121 | 0.959 | 0.044 | 0.328 | 0.037 |
| 18. 3,11diMe$C_{27}$ | 7.426 | 0.173 | 0.946 | 0.110 | 2.958 | 0.925 |
| 19. *x*$C_{28}$ | 1.081 | 0.083 | 0.404 | 0.024 | 1.237 | 0.158 |
| 20. 3,8diMe$C_{28}$ or 3,13diMe$C_{28}$ | 1.894 | 0.252 | 0.216 | 0.028 | 0.700 | 0.184 |
| 21. ? | 0.501 | 0.031 | 0.426 | 0.039 | 0.661 | 0.225 |
| 22. ? | 0.329 | 0.032 | 1.047 | 0.158 | 1.460 | 0.426 |
| 23. 6,10diMe$C_{28}$+6,18diMe$C_{28}$ | 0.514 | 0.032 | 0.683 | 0.104 | 0.651 | 0.076 |
| 24. 4,12diMe$C_{28}$+4,14diMe$C_{28}$ | 2.193 | 0.087 | 0.591 | 0.028 | 0.563 | 0.160 |
| 25. *n*$C_{29}$ | 1.566 | 0.121 | 4.221 | 0.330 | 2.823 | 0.443 |
| 26. 11Me$C_{29}$+13Me$C_{29}$+15Me$C_{29}$ | 4.419 | 0.177 | 5.405 | 0.205 | 8.464 | 0.518 |
| 27. 5Me$C_{29}$ | 0.944 | 0.062 | 1.405 | 0.070 | 0.933 | 0.054 |
| 28. 4Me$C_{29}$ | 1.456 | 0.117 | 12.708 | 0.356 | 6.318 | 0.662 |
| 29. 5,13di$C_{29}$ | 3.176 | 0.097 | 2.851 | 0.760 | 1.329 | 0.039 |
| 30. 3,11di$C_{29}$ | 4.159 | 0.147 | 2.828 | 0.108 | 7.047 | 1.496 |
| 31. *n*$C_{30}$ | 1.059 | 0.100 | 1.560 | 0.100 | 0.916 | 0.580 |
| 32. 13Me$C_{29}$+15Me$C_{29}$ | 1.196 | 0.049 | 0.724 | 0.059 | 0.124 | 0.091 |
| 33. ? | 2.315 | 0.118 | 5.832 | 0.361 | 4.266 | 0.662 |
| 34. 8-$C_{31}$:1+9-$C_{31}$: 1+10-$C_{31}$:1 | 1.174 | 0.053 | 2.129 | 0.122 | 1.825 | 0.224 |
| 35. ? | 0.435 | 0.083 | 1.077 | 0.049 | 0.872 | 0.343 |
| 36. 13Me$C_{31}$+15Me$C_{31}$ | 3.019 | 0.096 | 1.366 | 0.065 | 4.902 | 0.646 |
| 37. 8-$C_{32}$:1+10-$C_{32}$:1 | 0.788 | 0.047 | 10.840 | 0.347 | 8.732 | 0.891 |
| 38. *n*$C_{32}$ | 2.964 | 0.094 | 1.433 | 0.070 | 2.721 | 0.570 |
| 39. *x*Me$C_{32}$ | 0.932 | 0.646 | *t* | | *t* | |
| 40. 9-$C_{33}$:1 | 2.651 | 0.198 | *t* | | 0.610 | 0.084 |

*n* = Number of ants individually chromatographed (generally five per colony).

% ± S.D. = standard deviation.

*t* = traces.

(The total does not represent 100% because of the numerous small peaks.)

method with means UPGMA, [40]) was performed on the Nei Index distances between colonies. It confirms the results of the correspondence analysis; two main groups appear, with two subgroups in the "Provence" population (Fig. 4).

These results should be compared with a morphological distinction proposed by Forel [46]. He discriminated *C. cursor* var. *piliscapa* (region of Nîmes) from *C. cursor* by differences in pilosity. This difference was also detected by Bondroit [47] who named a new species *C. tibialis* (synonymous with *C. piliscapa* [48]). The diagnosis was later abandoned because pilosity

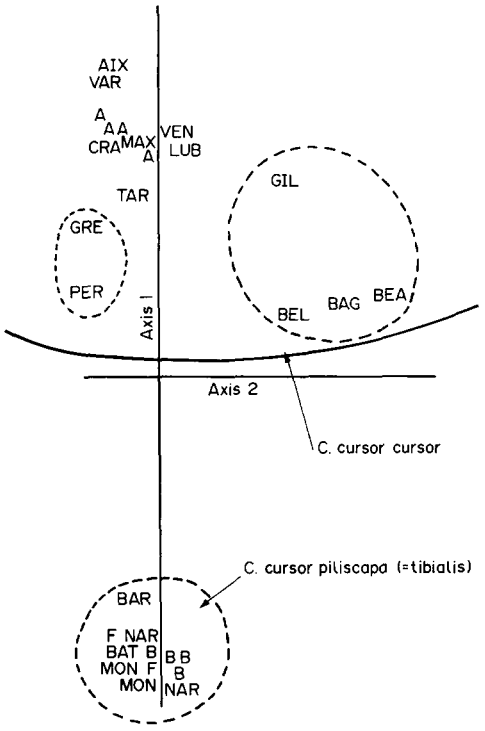


FIG. 2. PROJECTION ON THE TWO FIRST AXES OF CORRESPONDENCE ANALYSIS FOR VARIOUS SAMPLES. On the East of Rhône: A, Apt; BAG, Bagnol-sur-Cèze; BEA, Beaucaire; CRA, Crau; GIL, St-Gilles; LUB, Luberon; MAX, St-Maximin; TAR, Tarascon. On the West of the Rhône: B, Banyuls; BAR, Barcelone; BAT, col de la Bataille; F, Fort-Leucate; NAR, Narbonne; MON, Montpellier. Madrid Region: GRE, Gredos; PER, Pereguinos.

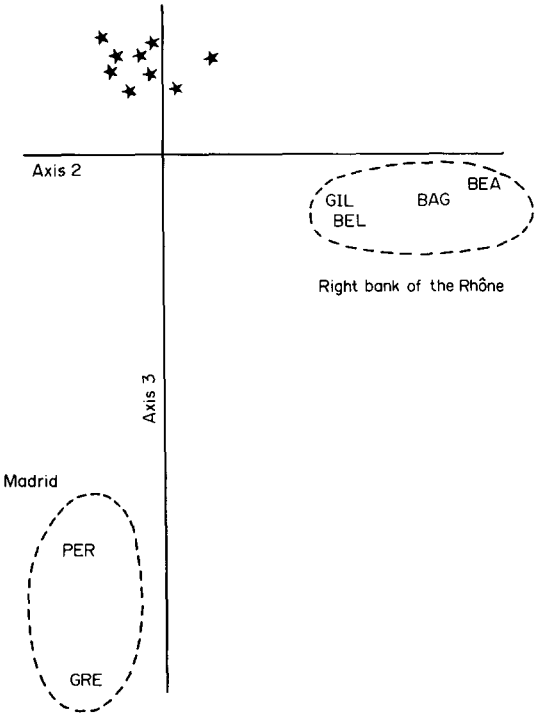


FIG. 3. PROJECTION ON THE AXES 2 AND 3 OF CORRESPONDENCE ANALYSIS—same samples as in Fig. 2. For legends see Fig. 2. All samples except those of the right bank of Rhône and Madrid are indicated by asterisks.

differences are not clear, but Nowbahari [45] showed with numerous samples that the ants of Provence generally have more hairs than those from Banyuls. Differences have recently been noticed in biometry, reproductive biology (Cagniant, personal communication), and male genitalia (Espadaler, personal communication). Agosti and Collingwood [47], in their key of European ant species, consider that *C. cursor* and *C. piliscapa* are good species which can be differentiated using the pilosity character. In the absence of a complete review, we prefer to speak of subspecies: *C. cursor cursor* (east of the Rhône) and *C. cursor piliscapa* (from the Rhône to the north of the Ebre in Spain). The Madrid population is also perhaps a different species which we can provisionally call *C. cursor "Madrid".*

The Nei identity index (*I*) was calculated between individuals from the various colonies (Table 3). Intracolony variation is low (0.94). In the same habitat intercolonial variation is not very different, but is significantly lower than intracolony variation (0.928 vs 0.94). As the distance between colonies increases the identity index decreases: 0.907 (<20 km) and 0.893 (>70 km) but the differences do not increase with the distance. For example, the ants from Barcelona can be closer to those of Montpellier (300 km) than ants from two locations in Pyrénées-Orientales which are separated by 20 km.

Between *C. cursor cursor* and *C. cursor piliscapa* the difference is important ($I=0.4$). Between *C. cursor cursor* and *C. cursor "Madrid"* the distance is smaller ($I=0.7$) but clearly remains below the level at which adoptions are possible, as will be seen.

*Relationship between colony aggressiveness and hydrocarbon patterns*

The results presented in [35] and new results [45]

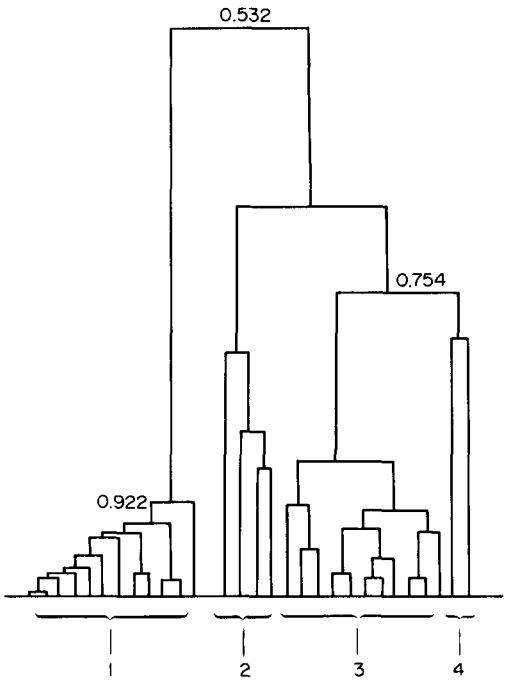


FIG. 4. CLUSTER ANALYSIS OF THE SAMPLES. 1, *C. cursor piliscapa*; 2, right bank of the Rhône; 3, *C. cursor cursor*; 4, Madrid.

TABLE 3. VARIATION OF CHROMATOGRAMS BETWEEN INDIVIDUALS

| Variation | *I* | *n* | *t* |
|---|---|---|---|
| Intracolony | 0.940 | 275 | |
| | (0.056) | | |
| Intercolony—same population | | | 2.326* |
| Same habitat | 0.928 | 301 | |
| | (0.068) | | 4.098** |
| Less than 20 km | 0.907 | 240 | |
| | (0.052) | | 1.659 NS |
| More than 70 km | 0.899 | 484 | |
| (1) | (0.082) | | |
| Intercolony—different populations | | | |
| *cursor cursor/cursor piliscapa* | 0.400 | 600 | |
| | (0.056) | | 22.641** |
| *cursor piliscapa/cursor "Madrid"* | 0.544 | 240 | |
| | (0.092) | | |
| *cursor cursor/cursor "Madrid"* | 0.714 | 220 | |
| | (0.074) | | 6.435** |
| Intercolony subpopulations | | | |
| Side of Rhône/*cursor cursor* | 0.667 | 374 | |
| | (0.105) | | |

*I*=Nei index distance (S.D.).
*n*=Number of measures.
*t*=Student's test *t*.
(1)=Sub populations from Madrid and banks of the Rhône excluded.
*=$P<0.02$, **=$P<0.01$.
NS=Not significant.

have been synthesized and related to hydrocarbon patterns. These data are based on the introduction of ants into alien colonies, compared with controls. The results were analysed only in terms of adoption or rejection after three days: the intruder is integrated into the resident society or is rejected into the foraging arena and either dies more or less rapidly, or is killed. Figure 5 shows that adoption never occurred when the similarity between colonies was lower than 0.8. We conclude that a good correlation exists between colony aggressiveness (regulation of the closure of societies) and hydrocarbon patterns. Lure experiments were necessary to reveal whether this correlation is really a causal factor.

*Hydrocarbon transfer*

Colonies of the two different populations (*C. cursor cursor* and *C. cursor piliscapa*) were used where intruders of the other population are always attacked and rapidly killed. The ants or the lures were introduced into the foraging arena of the recipient colony and observed for 10 min. Every 15 s, the behavior of resident ants was noted; two main categories of behavior were observed—aggressive or amicable behavior (Figs 6 and 7).

For these experiments we used live and dead (frozen) ants, neutral lures (ants washed with pentane) and pentane-washed lures covered with alien extracts. Ants were reintroduced into the original colony or placed in an alien colony.

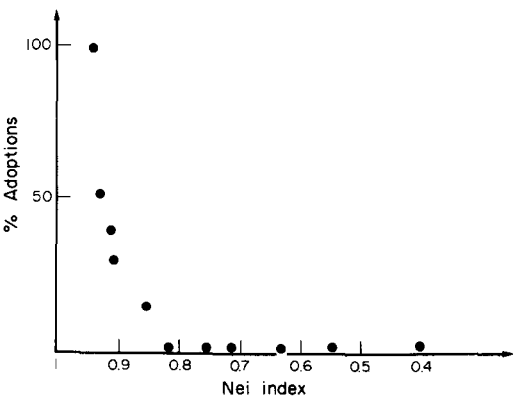


FIG. 5. RELATIONSHIP BETWEEN THE CLOSURE OF SOCIETIES (Indicated by the percentage of adoptions) AND THE SIMILARITY OF HYDROCARBON PATTERNS (Nei index).

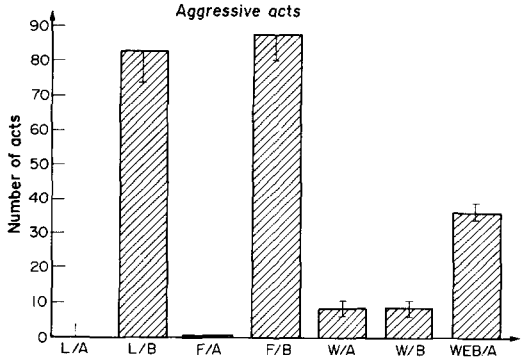


FIG. 6. NUMBER OF AGGRESSIVE ACTS OBSERVED IN VARIOUS SITUATIONS. L/A, live ant reintroduced into its colony A; L/B, live ant introduced into an alien colony B; F/A, frozen ant reintroduced into its colony A; F/B, frozen ant introduced into an alien colony B; W/A, washed ant reintroduced into its colony A; W/B, washed ant introduced into an alien colony B; WEB/A, washed ant treated with an extract of odor of colony B and reintroduced into its colony A.

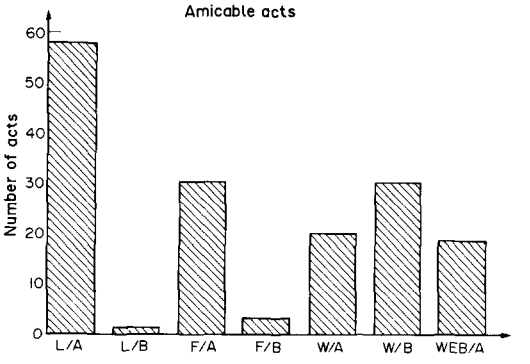


FIG. 7. NUMBER OF AMICABLE ACTS (see Fig. 6).

In control tests ants reintroduced into their original colony did not release any aggressive behavior. In alien colonies they were strongly attacked (88 aggressions in 10 min). We did not observe any significant difference between live and frozen ants at least before the first 10 min. This demonstrates that freezing maintains the properties of those substances involved in colony recognition. When the ant is washed with pentane, the level of attacks is diminished but never completely disappears. After being washed for a long period (12 h, less than 10 aggressions were observed, with no differences between control and alien colonies (8.5 vs 8.8 NS).

When neutral lures are covered with an extract from an alien colony, aggressiveness reappears (36.4). We noted 88 attacks with live ants, which indicates that the extract does not restore all the properties of colony recognition. Nevertheless it is possible to conclude that hydrocarbons have, at least in part, a role in colony recognition.

### *Chromatogram pattern of ants adopted by an alien colony*

Several authors have observed that callow ants are frequently adopted by alien homocolonial or heterospecific societies ([50]; for *C. cursor* see [51]). Thirty-eight workers were adopted by a colony originating from a different population. They were completely adopted, participating in the polyethism of the adoptive colony. After at least one month they were reintroduced into their native colony. In all cases they were immediately accepted. These ants can live in two colonies where the adults do not tolerate each other [52]. We analysed the hydrocarbons of some of these ants in comparison with the original colony and the adoptive colony (Table 4). The ants do not have their original pattern; the similarity index is 0.75 or 0.85 as against 0.98 for controls. They have acquired some characters of their adoptive colony; the index varies between 0.5 and 0.45. These ants are thus intermediate between the two colonies. It is noteworthy that in normal conditions an intruder with a hydrocarbon pattern lower than 0.8 is killed. In this situation the adopted ant is more different (0.5).

TABLE 4. IDENTITY INDEX BETWEEN ADOPTED ANTS Ad AND THEIR ORIGINAL COLONY A (Ad/A) OR THEIR ADOPTIVE COLONY B (Ad/B). For comparison distances between ants of the same colonies (A/A) or from different populations (A/B). Mean values (mini–maxi) for *n* data.

| | Ad/A | A/A | Ad/B | A/B |
|---|---|---|---|---|
| Ant no.1 | 0.850 | | 0.520 | |
| Apt/Banyuls | 0.788–0.881 | | 0.502–0.529 | |
| | $n=7$ | | $n=8$ | 0.448 |
| Ant no. 2 | 0.750 | | 0.550 | 0.4–0.494 |
| Banyuls/Apt | 0.697–0.761 | 0.981 | 0.521–0.640 | $n=16$ |
| | $n=6$ | 0.949–0.999 | $n=7$ | |
| Ant no. 3 | 0.684 | $n=8$ | 0.800 | 0.56 |
| Madrid/ | 0.704–0.664 | | 0.837–0.915 | 0.522–0.632 |
| Banyuls | $n=2$ | | $n=6$ | $n=8$ |
| Ants no. 4, 5 | 0.950 | | 0.962 | 0.958 |
| Banyuls/ | 0.906–0.985 | | 0.966–0.964 | 0.949–0.973 |
| Montpellier | $n=6$ | | $n=2$ | $n=8$ |

We hypothesize that learned factors are involved in colony recognition; the adults which adopt a callow are familiarized with the individual odor of this young ant which adsorbs on its cuticle some elements of the adoptive colony. It would be interesting to identify these substances which could play a crucial role in recognition.

This powerful test enables us to detect false adoptions. In one case (ant no. 3, Table 4) the ant was very different from its supposed nestmates (0.68 vs 0.98) and more similar to the supposed adoptive colony (0.8 vs 0.56). We can imagine that this ant has been introduced by error into a colony with a similar cuticular pattern. When adoptions are realized between comparable societies (e.g. Banyuls and Montpellier—ants nos 4 and 5), the results are not conclusive.

## Discussion

Hydrocarbon patterns seem to be a good index for intercolony aggressiveness. Bonavita *et al.* [24] on *Camponotus vagus*, Morel and Vander Meer [26] on *C. floridanus* obtained similar results. This is probably not a general situation, given that in the myrmicine species *Solenopsis invicta*, Obin [31] failed to find a correlation between aggressiveness and hydrocarbon patterns.

The problem of the origin of cuticular substances remains unsolved. Some authors suggest that they are produced by epidermal gland activity, but hydrocarbons are also found in post-pharyngeal glands [9, 53]. In *C. vagus* cuticular hydrocarbons for the whole body are comparable to those found in the head [54]. These authors suggest that the content of post-pharyngeal glands is deposited on the head from where it is dispersed over the whole body.

Our results indicate a predominant endogenous component in colony odor; ants introduced into alien colonies keep their original odor, but environmentally-acquired odors can also be adsorbed by the cuticle. This is the case of some myrmecophilous beetles. Errard and Jallon [55] observed that in artificial mixed colonies of different species some components can pass on to the other species.

Intercolony aggressiveness is regulated at least in part by differences in hydrocarbons. When colonies come from the same habitat and are kin because of budding dispersal [56], they have similar hydrocarbon patterns and show a low aggressiveness. Aggressiveness increases with the geographical distances between colonies, and differences in cuticular hydrocarbon patterns. A similar situation has been observed by Roisin *et al.* [57] for aggressive behavior in *Nasutitermes*.

The hydrocarbon differences between *C. cursor cursor* and *C. cursor tibialis* are very high ($I = 0.4$). In invertebrates, geographical populations show a high level of genetic identity for electrophoretic loci. In the *Drosophila willestoni* group, the Nei index was found to be 0.968 for geographical populations and 0.796 for subspecies [40]. Some preliminary data indicate an index of 0.4 between *C. cursor* and *C. hispanica* which is similar to the distance found between populations of *C. cursor* and indicates that *C. cursor* could be divided into at least two, and probably three species in the studied area (France and Spain). More data are needed, particularly on the morphology of the genitalia, to be sure of the species level of these populations which will be considered as species.

## Experimental

Colonies were collected from different regions and reared in the laboratory with the same food (mealworms and honey) for at least one month before experimentation.

*Chromatography*

Cuticular lipids were extracted by immersing one frozen worker in 2 ml of pentane for 5 min. The sample was concentrated under nitrogen and 2 µl injected in the chromatograph (GC) DELSI 300. The gas chromatograph was equipped with a flame-ionization detector and a 25 m × 0.22 mm capillary column (Chrompak CPSIL 5 WCOT). The temperature was programmed from 200 to 320°C at 3°C/min, after 20 s splitless. All GC analyses were conducted with the head and thorax of one ant. Gas chromatography–mass spectrometry (GC-MS) analyses were performed on a NERMAG R1010. The double bond positions were determined by alkylthiolation [36–38]. This method is more efficient than methoxymercuration which needs a lot of extract and gives heterogeneous chemical reactions. For alkylthiolation unsaturated fractions of TLC were solved into hexane. A DMDS solution and iodine acting as catalyst were added. The preparation was placed for 48 h in a cooker at 50°C. Iodine was then neutralized with sodium thiosulfate. The organic phase is extracted with hexane which is evaporated with nitrogen and finally solved in pentane for injection into the GC-MS (EI).

To compare the chromatograms we used the Nei index of identity [39] which is classically used in population genetics for enzymatic electrophoresis [40], but has also been used to compare chromatograms of terpenes in termites [41, 42].

$$I = \sum^n Xi * Yi / SQR\left(\sum^n Xi^2 * \sum^n Yi^2\right)$$

where $n$ = number of peaks, Xi = surface (%) of peak i for sample x, and Yi = surface (%) of peak i for sample y.

When patterns are strictly identical $I = 1$ and when patterns are totally different $I = 0$.

*Ethological tests*

For ethological tests we used the technique described in [35]: a worker or lure is introduced into the foraging arena of the colony. It is observed during 10 min and the behavior recorded every 15 s. Three lures were used: frozen workers (killed), workers washed with pentane to eliminate hydrocarbons, and washed workers impregnated with the extract of another colony. We distinguished aggressive acts (seizing with mandibles, dragging, projections of formic acid) and amicable acts (antennal inspection with closed mandibles, licking, trophallaxis, entering peacefully into the nest).

A total of 29 colonies were used, 27 from the Mediterranean region from the Var to Barcelone and two from the Sierra Mountains in the north of Madrid (Espadaler, personal communication and [43]. *C. cursor* has a wide geographical distribution but we did not collect Italian and eastern populations. Five workers per colony were used for individual chromatography.

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