# Regional multi-analyses of Argentine ant populations in southern Europe

Aggression and chemical heterogeneity within a putative global chemical and genetic homogeneity.

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## Objectives

Here we performed a regional multi-analyses to identify the expansion range of the Corsican supercolony, a new European supercolony (Blight et al. 2010) and to characterize its structure more explicitly. This is the first multi-analysis study conducted on Argentine ant populations in southern Europe. In view of the role of hydrocarbons and genetics in ant recognition, conducting joint behavioral, chemical and genetic analyses appeared to us a promising approach to understanding patterns of Argentine ant supercolony organization. This multidisciplinary approach was intended to allow us to formulate assumptions on the historical processes that may have produced this pattern, and its evolution.

## Methods

We collected nests of *L. humile* from 18 sites in southern Europe, ten colony fragments were collected in Corsica, six on the French mainland and two in Spain.

• We assessed the level of worker-worker aggression between all nesting sites using combinations of pairwise encounters consisting of confrontations between two groups of 100 workers that were competing for food and nesting space.

• We analyzed by gas chromatography/mass spectrometry (GC/MS) samples of one nesting site of each of the three European supercolonies. Further comparative analyses were performed using CHC extraction of individual workers (10 per nest) from each nesting site.

• DNA was extracted from 10 workers per nesting site (total 180) using the Puregene DNA Isolation Kit (Gentra Systems) analyzed at 12 microsatellite loci.

## Results

Members of this new supercolony were aggressive towards ants from the other two supercolony studied, i.e. the main European and the Catalonian supercolonies described by Giraud et al. (2002). Aggression between workers from the Corsican and the main European supercolonies varied from moderate to high (3.85 ± 0.06, n = 165), but was systematically high when workers from the Catalonian supercolony were involved (5 ± 0, n = 55). This behavioral demarcation was confirmed by chemical data (Fig. 1, 2; Table 1). Chemical proximity of members of the Corsican supercolony to members of the main supercolony was greater than to those of the Catalonian supercolony. Surprisingly, despite the concordance between chemical and behavioral groups, the main and the Corsican supercolonies were genetically undiscriminated. The central position of the nesting sites sampled here suggests that, from a genetic point of view, these nesting sites are related in some manner to the global supercolony described worlwide (van Wilgenburg 2010) (Fig. 3).

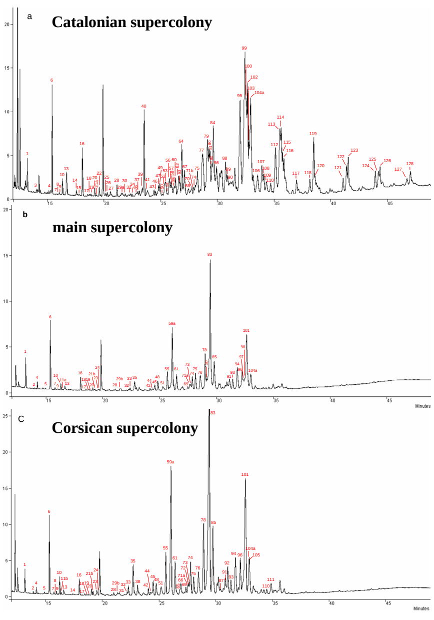


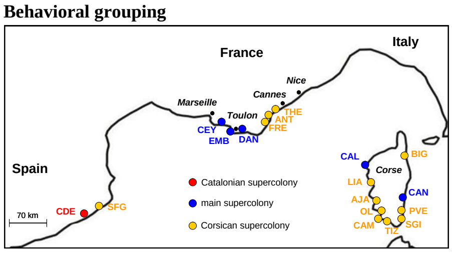

Fig. 1 Gas Chromatograms of total body wash of 30 workers of *Linepithema humile* from (a) Caldès d'Estrac (Catalonian supercolony), (b) Ceyreste (main European supercolony), (c) Fréjus (Corsican supercolony) and Giens (peace colony). Peak numbers correspond to the list of the identified cuticular hydrocarbons in Table 1.

Table 1. List of the identified cuticular hydrocarbons of workers from Caldès d'Estrac (Catalonian supercolony), Ceyreste (main European supercolony) and Fréjus (Corsican supercolony).

*Continued*

| Peak no | Compound | Caldès d'Estrac | Ceyreste | Fréjus | Peak no | Compound | Caldès d'Estrac | Ceyreste | Fréjus |
|---|---|---|---|---|---|---|---|---|---|
| 1 | *n*-C25 | + | + | + | 64 | 8,10,14-triMeC34 | + | | |
| 2 | 3-Me C25 | | + | + | 65 | 12,14- +12,16-diMe C34 | | | + |
| 3 | *n*-C26:1 | + | | | 66 | 8,12,20- +8,12,18-triMeC34 | | | + |
| 4 | *n*-C26 | + | + | + | 67 | 8,12,16-triMeC34 | + | | |
| 5 | 2-Me C26 | | + | + | 68 | x,y,z-triMeC34 | + | | |
| 6 | *n*-C27 | + | + | + | 69 | 4,14-diMeC34 | | + | + |
| 7 | 13-MeC27 | + | + | + | 70 | 6,12,20-triMeC34 | + | | |
| 8 | 7-MeC27 | + | | + | 71a | 6,14,18-triMeC34 | | + | + |
| 9 | 5-MeC27 | + | + | + | 71b | 11,x,y,z-tetraMeC33 | + | | |
| 10 | 3-MeC27 | + | + | + | 72 | 6,14,18-triMeC34 | | | + |
| 11a | 5,15-diMeC27 | | + | | 73 | *n*-C35 | + | + | + |
| 11b | 5,11-diMeC27 | | | + | 74 | 4,14,18-triMe | | + | + |
| 12 | *n*-C28 : 1 | + | | | 75 | 13- + 15- + 17-MeC35 | + | + | + |
| 13 | *n*-C28 | + | + | + | 76 | 13,17- + 15,19-diMeC35 | | + | + |
| 14 | 4-MeC28 | + | | + | 77 | 11,19- + 13,19- + 15,19-diMeC35 | + | | |
| 15 | 2-MeC28 | + | | | 78 | 5,15-diMeC36 | | + | + |
| 16 | *n*-C29 | + | + | + | 79 | 13,17,21-triMeC35 | + | | |
| 17 | 11- + 13- + 15-MeC29 | + | + | + | 80 | 9,13,15-triMeC35 | + | + | |
| 18 | 7-MeC29 | + | + | + | 81 | 7,11,15-triMeC35 | + | | |
| 19 | 5-MeC29 | + | + | + | 82 | *n*-C36 | + | | |
| 20 | 3-MeC29 | + | + | + | 83 | 5,13,17- +5,13,15-triMeC35 | | + | + |
| 21a | 5,9-diMeC29 | + | | | 84 | 7,11,15,17-tetraMeC35 | + | | |
| 21b | 5,11-diMeC29 | | + | + | 85 | 14,16-diMeC36 | | + | + |
| 22 | *n*-C30 | + | | | 86 | 12,14-diMeC36 | + | | |
| 23 | 14- + 16-MeC30 | | + | + | 87 | 6,14- +6,18-diMeC36 | | | + |
| 24 | 12-MeC30 | | + | + | 88 | x,y,z-triMeC36 | + | | |
| 25 | 8-Me C30 | + | | | 89 | 8,12,20-triMeC36 | + | | |
| 26 | 4-MeC30 | + | | | 90 | 7-MeC37 | + | | |
| 27 | 2-MeC30 | + | | | 91 | 6,14,18-triMeC36 | | + | + |
| 28 | *n*-C31 | + | + | + | 92 | 4,14,18 triMeC36 | | | + |
| 29a | 13- + 15-MeC31 | + | | | 93 | 13- + 15- + 17- +19-MeC37 | | + | + |
| 29b | 11- +13- + 15-MeC31 | | + | + | 94 | 13,15- + 15,19- +17,19-diMeC37 | | + | + |
| 30 | 8,10,x,y-TetraMeC30 | + | | | 95 | 13,17- + 15,21- + 13,19-diMeC37 | + | | |
| 31 | 5-MeC31 | | | + | 96 | 5,15-diMeC37 | | + | + |
| 32 | 3-MeC31 | + | + | + | 97 | 9,13,15-triMeC37 | | + | |
| 33 | 5,13- + 5,15-diMeC31 | | + | + | 98 | 7,13,15-triMeC37 | | + | |
| 34 | 5,11- diMeC31 | + | | | 99 | 11,19,23-triMeC37 | + | | |
| 35 | 5,13,15 - + 5,13, 17-triMeC31 | | + | + | 100 | 9,13,15-triMeC37 | + | | |
| 36 | 10- + 12-MeC32 | + | | | 101 | 5,15,17-triMeC37 | | + | + |
| 37 | 5,7,11-triMeC31 | + | | | 102 | 7,13,15-triMeC37 | + | | |
| 38 | 14,18- + 14,16-diMeC32 | | | + | 103 | 9,13,15,18-tetraMeC37 | + | | |
| 39 | 8,12- +8,14-diMeC32 | + | | | 104a | 14,16- +14,18-diMeC38 | | + | + |
| 40 | 8,10,14-triMeC32 | + | | | 104b | 7,11,15,19-tetraMeC37 | + | | |
| 41 | 6,10,14-triMeC32 | + | | | 105 | 14,16- +14,18-diMeC38 | | | + |
| 42 | 4,14- +4,16-diMeC32 | | + | + | 106 | 12,18- + 14,18-diMeC38 | + | | |
| 43 | *n*-C33 | + | | | 107 | 12,14,20- + 12,16,20-triMeC38 | + | | |
| 44 | 6,14,16-triMeC32 | | + | | 108 | 8,12,20-triMeC38 | + | | |
| 45 | 4,14,16-triMeC32 | | + | + | 109 | 8,12,16,20-tetraMeC38 | + | | |
| 46 | 9,13,17,19-tetraMeC31 | + | | | 110 | 13 + 15 + 17 +19-MeC39 | + | | + |
| 47 | 10,16,22,24-tetraMeC32 | + | | | 111 | 13,17- + 17,19-diMeC39 | | | + |
| 48 | 13- + 15- + 17-MeC33 | | + | + | 112 | 11,17- + 13,17- + 13,19-diMeC39 | + | | |
| 49 | 11- + 13- + 15-MeC33 | + | | | 113 | 11,17,19- +13,17,19-triMeC39 | + | | |
| 50 | 8,10,x,y-TetraMeC32 | + | | | 114 | 9,13,17-triMeC39 | + | | |
| 51 | 11,19- + 11,13-diMeC33 | | + | + | 115 | 7,13,17- + 7,11,17-triMeC39 | + | | |
| 52 | 11,15- + 13,15-diMeC33 | + | | | 116 | 9,13,17,19-tetraMeC39 | + | | |
| 53 | 9,13-diMeC33 | + | | | 117 | 12,16,22-triMeC40 | + | | |
| 54 | 7,13- +7,11-diMeC33 | + | | | 118 | 13,19- + 13,21-diMeC41 | + | | |
| 55 | 5,15- + 5,17-diMeC33 | | + | + | 119 | 13,19,23-triMeC41 | + | | |
| 56 | 5,11- diMeC33 | + | | | 120 | 9,13,19-triMeC41 | + | | |
| 57 | 7,11,15-triMeC33 | + | | | 121 | x,y-diMeC43 | + | | |
| 58 | 7,11,13-triMeC33 | + | | | 122 | 13,19,23-triMeC43 | + | | |
| 59a | 5,13,15-triMeC33 | | + | + | 123 | 9,13,21-triMeC43 | + | | |
| 59b | 5,9,13-triMeC33 | + | | | 124 | x,y-diMeC45? | + | | |
| 60 | 7,11,13,17-tetraMeC33 | + | | | 125 | x,y,z-triMeC45 ? | + | | |
| 61 | 14,16- + 16,18-diMeC34 | | + | + | 126 | x,y,z-triMeC45 ? | + | | |
| 62 | 12,14- +12,16-diMe C34 | + | | | 127 | x,y,z-triMeC47 ? | + | | |
| 63 | 8,12- +8,14-diMeC34 | + | | | 128 | x,y,z-triMeC47 ? | + | | |
| | | | | | | total | 93 | 49 | 57 |

**Behavioral grouping**




**Chemical grouping**

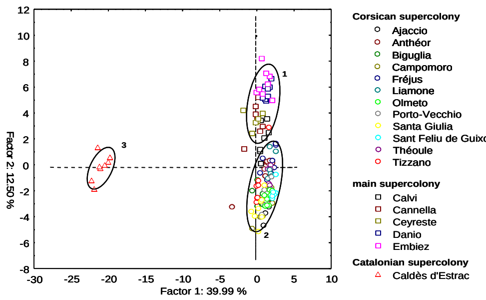



**Fig. 2** Principal Component Analysis based on cuticular profiles of *Linepithema humile* workers from the 18 nesting sites (n = 153). The projection was performed on factorial plane 1-2. Axis 1 accounts for 39.99 % of total variance and axis 2 for 12.50 %. Results of *K*-means cluster analyses, group 1: the main supercolony (CAL, CAN, CEY, DAN, EMB), group 2: the Corsican supercolony (ANT, AJA, BIG, CAM, FRE, LIA, PVE, SFG, SGI, TIZ, THE) and group 3: the Catalonian supercolony (CDE). Nesting sites are assigned different symbols following results of behavioral tests.

**Genetics**

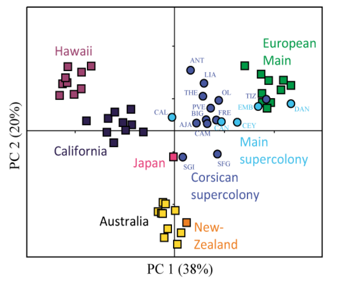



**Fig. 3** Principal Component Analysis based of microsatellite allele frequencies calaculated on nests that may have a common history. We included in the analysis all the nesting sites of the main and the Corsican supercolonies of the present study (represented by dots) as well as all the nests of the world wide supercolonies studied in Vogel et al. (2010) sharing the same haplotype (squares).Note that the sampling was performed at different scale depending of the supercolonies (see Vogel et al 2010 for more details) which may explain the differences in the extend in the graph of the supercolonies. The percentage of variance explained is given in brackets.

## Conclusions

▪ This study confirms the existence of a distinct Corsican supercolony with a distribution range of at least Corsica, southeastern France and one location in Spain.

▪ What we report here is, to our knowledge, the first recorded aggression between supercolonies that are genetically undiscriminated, at least for the Argentine ant.

▪ We demonstrate that genetically similar nests at microsatellite markers do not necessarily belong to the same supercolony.

▪ In the light of our results, the recent identification of a global supercolony formed by the largest intercontinental supercolonies, which are viewed as genetically and chemically similar, needs to be considered with caution.

▪ The process of nestmate recognition that behaviorally segregate the Corsican supercolony from the main supercolonies remains unclear.

▪ Genetic data also raise questions on the origin and evolution of this regional pattern.

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