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Evolutionary consequences of deception: Complexity and informational content of colony signature are favored by social parasitism

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Abstract Nestmate recognition codes show remarkable chemical complexity, involving multiple biochemical pathways. This complexity provides the opportunity to evaluate the ecological and social conditions that favor the evolution of complex signaling. We investigated how the chemical signatures of three populations of the social paper wasp *Polistes biglumis* differed in terms of concentration of hydrocarbons, proportions of branched hydrocarbons and overall variation. We tested whether the variation in chemical signatures among populations could be explained by the prevalence of social parasites or whether this was just an effect of local abiotic conditions which influenced the composition of the hydrocarbon cuticular layer. We studied the chemical signature in three populations in which obligate social parasites differed in the selection pressures they imposed on host populations. Within each population, we restricted our analyses to non-parasitized hosts, to avoid potential short-term effects of parasite presence on the host chemical signatures. We found that host colonies in parasitized populations had more diverse profiles than the parasite-free population. Moreover, the overall concentration of hydrocarbons and the relative proportion of branched hydrocarbons were larger in the parasitized populations, relative to the non-parasitized one. This is to our knowledge the first evidence in favour of the hypothesis that different traits in the host chemical signatures as a whole undergo evolutionary changes resulting from directional or balancing selection imposed by social parasites. We conclude that obligate social parasites act as 'engines of diversity' on host chemical signatures and operate in favor of a geographic mosaic of diverging communication codes [*Current Zoology* 60 (1): 137–148, 2014].

Keywords *Polistes*, Brood parasitism, Cipher, Nestmate recognition, Hydrocarbons, Geographic mosaic, Crozier's paradox

Encrypted communication involves the transfer of simple information encoded in complex ways. This is the case, for example, of ciphers, i.e., secret and complex codes used by closed social units. Cipher complexity arises as a result of antagonistic interactions and ongoing coevolution between code makers and code breakers (Singh, 2000). Social insect colonies are closed social units, which defend their attractive resources, such as food stores, eggs, broods, and protected shelters from intruders (Hölldobler and Wilson, 1990). Colony members protect their resources by discriminating unwanted intruders from nestmates, and rejecting intruders (Fletcher and Michener, 1987; Guerrieri et al., 2009). Nestmate/non-nestmate discrimination processes occur via recognition codes: social insects discriminate against individuals lacking their own colony signature (Gamboa, 2004; Howard and Blomquist, 2005). In this respect, social insects are code makers that use chemical codes to encrypt colony affiliation. Social insect colonies also include complex social structures, which involve both sophisticated social organizations and an efficient workforce. These resources are especially targeted by social parasites: insects which rely on the social organization and workforce of host species to rear their own offspring (Wilson, 1971). Often, social parasites invade host colonies and integrate stably within their social structures. Usually, social parasites break the host colony recognition codes, either by concealing their true identity to hosts (chemical insignificance, Lenoir et al., 2001; Uboni et al., 2012) or by breaking into their host recognition codes and "copying" them (chemical mimicry or camouflage, Bagnères and Lorenzi, 2010; Nash and Boomsma, 2009). However, host colony recognition codes are often very elaborate. In fact, the content of their messages is simple ("I am affiliated with colony x"), but the way information is

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encrypted is very complex (Blomquist and Bagnères, 2010; Richard and Hunt, 2013). This complexity may have arisen as a result of antagonistic interactions with code breakers. The harder it is to counterfeit colony recognition codes, the lower the probability that parasites can threaten colony integrity. Therefore, as for ciphers, colony affiliation codes are continuously under attack by social parasites and are potentially the object of an ongoing coevolution, whereby parasites select hosts for complex codes and hosts select parasites for the ability to break codes.

As a result of this co-evolutionary process, social parasites and their hosts have commonly evolved complex suites of traits. The parasites, for example, often use many strategies to overcome host colony defenses and intercept host communication codes; these strategies have been widely investigated (Lenoir et al., 2001; Lorenzi, 2006; Bagnères and Lorenzi, 2010). The hosts often have complex nestmate recognition mechanisms that efficiently (albeit not completely) identify unwanted insects in the colony (Gamboa 1986, 2004; Pickett et al., 2000; Lorenzi, 2003). Fewer studies have been undertaken on host defense, but these studies have suggested that hosts evolve morphological, behavioral, and chemical adaptations in the coevolutionary arms race with their social parasites (e.g. Foitzik et al., 2009; Ruano et al., 2011; Lorenzi and Thompson, 2011). Similar complexity of defense measures and counter-measures has been found in other kinds of parasitism, such as brood parasitism, where the focus of selection in those interactions has been more on traits such as egg polymorphism, nest defense and egg or chicks rejection (Lathi, 2005; Rothstein, 1982; Soler and Soler, 2000; Spottiswoode and Stevens, 2011; Colombelli-Négrel et al., 2012).

In social insects, colony affiliation is advertized with chemical signatures composed mainly of cuticular hydrocarbons. These are lipid substances that primarily serve as anti-desiccation agents; they form a hydrophobic layer over the cuticle of insects, preventing water loss via transpiration through the cuticle (Blomquist and Bagnères, 2010). The layer works best at preventing water loss at lower temperatures and when it is mainly composed of straight-chain alkanes, as insertion of double bonds and methyl-branching significantly lower its anti-desiccation properties (Gibbs 1998, 2002; Gibbs and Rajpurohit, 2010; Hefetz, 2007). In contrast, the communicative function of cuticular hydrocarbon blends is likely to be enhanced by the presence of molecules with complex shapes, such as alkenes and methyl-branched compounds (Dani et al., 2001; Lorenzi et al., 2011). Here we tested two competing hypotheses based on the dual role of cuticular hydrocarbons as agents of anti-desiccation and agents of communication. We reasoned that the communication role of hydrocarbons could assume even greater importance when social insects are under attack by social parasites. Social parasites employ different chemical strategies that facilitate their integration into host colonies. Often, social parasites mimic the colony recognition codes of their hosts (chemical mimicry, i.e., they exhibit recognition cues that match those of their hosts, Lenoir et al., 2001; Bagnères and Lorenzi, 2010). The better the parasite mimics the host's cuticular signature, the faster the adoption by host colonies (Nash et al., 2008). Parasites can also elude recognition (chemical insignificance, i.e., possess low concentration of recognition cues, Lenoir et al., 2001). The lower the amount of recognition cues, the shorter the time hosts spend attacking (Cini et al., 2009). Host populations infested by such parasites would have to mount defenses on the same ground, endowing themselves with distinctive hydrocarbon signatures, in terms of absolute concentrations of hydrocarbons, proportions of branched hydrocarbons and/or profile variation. Indeed, increasing the concentrations of recognition cues is a simple way to enhance the efficacy of chemical communication (Steiger et al., 2011). There might not be such an advantage in less infested populations, as hydrocarbons, and especially branched hydrocarbons, are costly to produce and/or maintain and branched hydrocarbons deteriorate the anti-desiccation function (Hefetz, 2007, LeConte and Hefetz, 2008).

Therefore, we tested whether differences in the hydrocarbon signatures between populations of social insects could be explained by parasite prevalence or whether they were solely an effect of local abiotic conditions such as temperature and humidity.

If social parasites impose selection on hosts for the evolution of distinctive hydrocarbon signatures, we predicted that social insects from populations with high parasite prevalence would display higher concentration of hydrocarbons, higher proportions of branched hydrocarbons and higher profile variation than less infested populations. Such variations would indicate that social insect populations under high parasite prevalence are under selection for enhancing the communicative role of their cuticular hydrocarbon blends. Alternatively, if selection on hydrocarbon signatures is mainly imposed by abiotic factors (such as air temperature or rainfall regime), we predicted that hydrocarbon signa-

tures would vary in a consistent way with local climatic conditions, i.e., by enhancing the anti-desiccation role under higher temperature and/or lower rainfall regimes. Accordingly to the latter hypothesis, we expected populations under relatively higher temperature-regimes to have higher concentrations (total amount) of hydrocarbons and lower proportions of branched hydrocarbons. We also expected populations with relatively high rainfall-regimes to have lower concentrations of hydrocarbons and higher proportions of branched hydrocarbons. Finally, we expected that temperature and rainfall regimes did not affect within-population variation in profile (see Table 1 for a schematic presentation of predictions under the two scenarios).

1 Materials and Methods

1.1 Study species

Our model species was the social wasp *Polistes biglumis*. These wasps discriminate nestmate from non-nestmates using cuticular hydrocarbons (Lorenzi et al., 1997). In this way they reject conspecific facultative social parasites attempting nest usurpation and defend their colonies from robbery by conspecifics and cleptoparasites (Lorenzi and Cervo, 1995; Uboni and Lorenzi, 2013). Populations of *P. biglumis* can also be infested by a congeneric, obligate social parasite *P. atrimandibularis*, which overcome host recognition using both chemical insignificance and chemical mimicry (Bagnères et al., 1996; Lorenzi and Bagnères, 2002; Uboni et al., 2012).

Polistes biglumis is a free-living social wasp with annual colonies. Each colony is founded in late spring by a single foundress which builds the nest, lays eggs and rears her brood solitarily during a long founding phase (Lorenzi and Turillazzi, 1986). Foundresses efficiently recognize their nestmates and their eggs (Lorenzi et al., 1999; Lorenzi and Filippone, 2000). As other *Polistes* wasps, foundresses establish their colony signature by marking nests with their cuticular signatures, which is composed of > 60 hydrocarbons (Lorenzi, 1992; Lorenzi et al., 1997, 2011; Costanzi et al., 2013). During the founding phase, colonies can be invaded by the obligate, workerless, social parasite wasp *Polistes atrimandibularis*. A single parasite peacefully enters a host colony, subdues the host foundress and lives in the host colony until the end of the summer, inhibiting foundress reproduction and reducing her fitness (Cervo et al., 1990; Lorenzi et al., 1992; Cervo and Lorenzi, 1996a). Parasites elude host recognition using a composite strategy that includes chemical insignificance and chemical mimicry. At host-nest invasion, these parasites have hydrocarbon signatures different from their hosts, but quantitatively poor (chemical insignificance) (Lorenzi and Bagnères, 2002). As they live in host colonies, parasites change their hydrocarbon signatures; they lose parasite-specific compounds and enrich in host-specific compounds, and especially in branched-hydrocarbons (chemical mimicry) (Bagnères et al., 1996; Lorenzi et al., 2004; Uboni et al., 2012). As a result, social parasites integrate into host colonies so well that hosts discriminate between their own parasites and alien parasites, and attack the latter only (Lorenzi, 2003). We do not know whether the acquisition of host-specific compounds occurs passively or whether it involves the expression of previously-silent biosynthetic pathways. Whatever the underlying mechanism, mimicry of host-specific compounds on the part of parasites may trigger chemical arms races with hosts. We expect that populations subject to obligate parasitism have enhanced the communication role in their signatures, but parasite-free populations will not lack it. Indeed, hydrocarbon signatures also allow the identification of conspecific cheaters, i.e., facultative social parasites which are common in this species (Lorenzi and Cervo, 1995; Cervo and Lorenzi, 1996b) and overmark host-nest signatures with their own signatures (Lorenzi et al., 2007, 2011).

1.2 Study populations

P. biglumis populations have a patchy distribution in the Alps, due to geomorphology of their mountain habitat and to large areas unsuitable for nesting (rocky areas and forests). These geographic barriers contribute to separate populations together with foundress philopatry (Reeve, 1991; Hunt, 2007) and local matings (Seppä et

Table 1 Expectations based on the contrasting hypotheses that either biotic or abiotic factors affect traits that contribute to colony signature, in terms of concentration, composition or quantitative variation of the cuticular hydrocarbon blends

Sources of selection		Colony signature traits		
		Concentration of hydrocarbons	Proportion of branched hydrocarbons	Quantitative profile variation
Biotic	parasite prevalence	positive correlation	positive correlation	positive correlation
Abiotic	high temperature	positive correlation	negative correlation	unaffected
	high rainfall	negative correlation	positive correlation	unaffected

al., 2011). Indeed, populations differ in life-history traits, as a result of both local climate and social parasite pressures (Fucini et al., 2009, 2013; Lorenzi and Thompson, 2011). *P. biglumis* populations are small and scattered in the Alps above 1200 m a.s.l., where other host species are very rare. *P. biglumis* was the only free-living *Polistes* wasp nesting at the study sites. Therefore, where the social parasite *P. atrimandibularis* was present, there were no alternate hosts to *P. biglumis*.

Sampling on multiple mountain field-populations is time-consuming and longitudinal field-data on parasite prevalence are rarely available for social insects. Therefore, we had to restrict our analysis to three populations where the prevalence of the social parasite *P. atrimandibularis* across years was known. The sampled populations have different social-parasite prevalence: Montgenèvre, where social parasitism is high (23.5% parasitized colonies over 5 years), Ferrere, where it is intermediate (5.6% parasitized colonies over 5 years), and Carì, where parasites are absent (no parasite has been sampled since we discovered this population in 2006) (Lorenzi and Thompson, 2011). Populations were between 1600–1900 m a.s.l. and 70–250 km apart. At the moment, there is no information on genetic diversity in the study populations which might be useful to infer the demographic history of these populations and identify and date past population-size changes (expansions or bottlenecks).

1.3 Parasite prevalence

Parasite prevalence in each population was determined through field censuses. From the early founding phase (May–June) until the end of the nesting cycle, during each of 3–5 years, we monitored the breeding attempts of 1648 individually marked foundresses (Montgenèvre, years: 2004–2008: n = 498; Ferrere, years: 2004–2008: n = 464; Carì, years 2006– 2008: n = 170 foundresses) (details in Lorenzi and Thompson, 2011). Each population was visited every 2–10 days checking for foundress presence and whether the colony had been invaded by a *P. atrimandibularis* parasite female. When we found parasites, we individually marked them. Prevalence (percentage of colonies invaded by *P. atrimandibularis* parasite females) was roughly constant per population and year (Lorenzi and Thompson, 2011).

1.4 Sample collection

We collected foundresses in the pre-emergence period, i.e., when the single foundress was the only adult wasp on the nest (Montgenèvre: n = 8, Ferrere: n = 10, Carì: n = 11 foundresses) (date: July 8–20, 2009; July 13–22, 2010). We were interested in differences between populations in hydrocarbon signatures due to evolutionary changes. However, hydrocarbon signatures could be adjusted not only in evolutionary terms, where conditions encountered in subsequent generations select for optimal signatures, but also in terms of phenotypic plasticity, where enslaved foundresses make short-term adjustments in their signature as a response to enslavement. Therefore, none of the collected foundresses came from parasitized colonies or from colonies usurped by conspecific females, so that we could exclude short-term, parasite-mediated modifications of host hydrocarbon signatures.

1.5 Chemical analyses

Foundresses were killed by freezing and weighed (precision balance Precisa 125A). We extracted their cuticular compounds by separately suspending each foundress in 1-ml pentane for 60 sec. An internal standard (n-C20) was added to each extract to quantify compounds.

For quantitative analyses, we analysed samples using gas-chromatography with flame ionization detection (AGILENT 6850). Two μl of each extract (which included 800 ng of n-C20) were injected in the gas-chromatograph equipped with a Chrompack CPSIL5 WCOT CB nonpolar-capillary-column (30 m length × 320 μm internal diameter × 0.25 μm thickness of stationary phase) (carrier gas: helium, 1 bar, 50 ml/min flux). Oven temperature increased from 70°C to 150°C at a 30°C/min rate and from 150°C to 320°C at a 5°C/min rate (10 min at 320°C final temperature). Results were registered in an Agilent-ChemStation. The area of each peak was converted to its proportional contribution to total peak area in that sample. The compounds in the profiles were determined via GC-MS as in previous work (Bagnères et al., 1996; Lorenzi et al., 1997; Uboni et al., 2012) and checked for each population.

1.6 Meteorological data: Air temperature and precipitation

Meteorological data were obtained from weather stations (Montgenèvre and Ferrere: ARPA; Carì: METEOMEDIA AG). The closest weather stations were at lower altitudes than the populations themselves, so a correction was applied to account for the temperature drop owing to altitude increase (lapse rate: 0.6°C each 100m) (Körner, 2007). For each population, we calculated temperature regime as the average among the maximum air-temperature (°C) recorded each day during the 30 days preceding foundress collection, and rainfall regime as the average precipitation (rain mm/day) during the same period. This was done for

both years of collection (data averaged across years).

1.7 Statistical analyses

1.7.1 Differences in concentrations

Following procedures described elsewhere (e.g. Lenoir et al., 2001, Lorenzi et al., 2011, Costanzi et al., 2013, Quezada-Euán et al., 2013), we measured the total concentration of hydrocarbons for each foundress as the sum of all peak areas relative to the area of *n*-C20. We divided these measures by the body weight of each foundress (ng of hydrocarbons/mg of body weight). This measure reflects the concentration of hydrocarbons on the cuticular surface. Differences in the total concentration of hydrocarbons among populations were analyzed using a Linear Mixed effects Model (LMM) (R package lme4, Bates et al., 2013), using identity link function, with population as the fixed factor and year a random factor. Concentration data (and model residuals) followed the assumptions of normality and homoscedasticity. As our model contained only one fixed factor, model comparison or simplification was not feasible; we tested the significance of the population effect using the function Anova from the package lmerTest (Kenward-Roger approximation for the denominator degrees of freedom) (Kuznetsova et al., 2013).

1.7.2 Differences in proportions

The epicuticular hydrocarbons of *P. biglumis* are either linear alkanes or methyl-branched alkanes (Lorenzi et al., 1997, 2011). Traces of alkenes may be present in negligible concentrations (< 0.5%) (Uboni et al., 2012). Therefore, differences between populations in the proportions of branched hydrocarbons (proportional contribution of all branched hydrocarbons to total peak area in each sample) were analyzed using a Generalized Linear Model (GZLM) for binomial distribution data (hydrocarbons were either linear or methyl-branched) and logit link function. We first fitted a Generalized Linear Mixed effects Model (fixed factor = population, random factor = year) in R (package glmmML, Broström, 2013), but as the deviance associated with the random factor was 0 (test of null hypothesis of no random effect, p = 0.5), we reverted to a GZLM (function glm from package stats, family = binomial, link = logit, fixed factor = population). Tukey's contrasts for multiple comparisons were used as post-hoc tests.

1.7.3 Associations between hydrocarbon signature traits and parasite prevalence, rainfall or air temperature

When testing for associations between hydrocarbon signature traits and biotic (or abiotic) variables, we used a directional test. Ordered Heterogeneity test (OH test) allows testing against simply ordered alternative hypotheses in the context of nondirectional tests (Rice and Gaines, 1994). This procedure tests for between-population differences while taking an a priori expectancy into account, i.e., it combines an existing k-sample heterogeneity test with the Spearman-rank-order-correlation coefficient between observation and expectancy. Here, it included the *a priori* expectancies that we summed up in Table 1. These expectancies were used to test the relationships between the two variables (concentrations of hydrocarbons and proportions of branched hydrocarbons) and the sources of selection (parasite prevalence, air temperature and precipitation). Therefore, we performed six OH tests, after ranking the three populations for 1) parasite prevalence; 2) temperature regime and 3) rainfall regime (Table 2). We first tested whether there were significant differences between populations in hydrocarbon concentrations and branched-hydrocarbon proportions using LMMs or GZLMs, and then tested whether population means were ordered in the expected direction using OH test. We computed the r_sP_c statistic values as the product $r_s(1 - P_c)$ where r_s was the Spearman's-rank-correlation coefficient between each variable and the source of selection and P_c was a P-value from the model. Critical values for the r_sP_c statistic are given in Rice and Gaines (1994) for selected alpha values (exact P-values cannot be computed).

The two variables (total concentrations of hydrocarbons and proportions of branched hydrocarbons) were not significantly correlated with each other (Spearman's-correlation test, Montgenèvre: rho = 0.190, P = 0.651; Ferrere: rho = 0.384, P = 0.273; Carì: rho = 0.245, P = 0.467).

1.7.4 Multivariate dispersion of hydrocarbon profiles within populations

We tested the variation of hydrocarbon signature within and between populations using an analysis of multivariate homogeneity of group dispersion on the entire hydrocarbon signature (64 peaks), following Anderson's PERMDISP procedure (Anderson, 2006). This is a multivariate analogue of Levene's test for homogeneity of variances used to evaluate beta diversity among different species assemblages/areas (Anderson et al., 2006). Bray-Curtis dissimilarity measures on the relative proportions of hydrocarbons were calculated for each population, and submitted to betadisper, a function of the R package vegan (Oksanen et al., 2013) that implements PERMDISP2.

We visualized the relationships (and dispersion) between the chemical profiles of foundresses from the

three populations by performing a two-dimensional Non-Metric Multidimensional Scaling (NMDS) (with 95% confidence ellipses) with the program PAST version 2.17 (Hammer et al., 2001).

2 Results

The chemical signatures of *P. biglumis* foundresses in the three populations were complex blends including more than 60 peaks representing homologous series (C23-C35) of linear and methylbranched alkanes (confirming previous data from a population, Lorenzi et al., 1997; Uboni et al., 2012) (Fig.1).

Parasite prevalence was the highest in Montgenèvre, where on average 23.5% of colonies (1 every 5) were invaded by social parasites. It was intermediate in Ferrere, where 5.6% of the colonies (1 every 20) were parasitized. Parasites were absent in Carì (Table 2, Fig. 2A).

2.1 Differences in concentration and proportions

2.1.1 Concentration of hydrocarbons

The differences in the concentration of cuticular hydrocarbons between populations were significantly associated with parasite prevalence (OH test: r_sP_c = 0.9991, k=3, $P < 0.01$) and rainfall regime (OH test: r_sP_c = 0.9991, k=3, $P < 0.01$), but not with temperature (OH test: r_sP_c = 0.49955, k=3, $P > 0.05$) (Table 2).

Foundresses differed significantly in hydrocarbon concentration between populations (LMM fit via lme4 random effect: σ(year) = 2.851, σ(residual) = 46.301; fixed effect: population: $F_{2,25.37}$ = 12.538, $P < 0.001$). Foundresses from Montgenèvre, the most parasitized population, had on average higher hydrocarbon concentrations than those from Ferrere (Tukey's test, $P < 0.001$) and Carì, where parasites were absent ($P < 0.001$) (Fig. 2B).

2.1.2 Proportion of branched hydrocarbons

The differences in the proportion of branched hydrocarbons were significantly associated with parasite prevalence (OH test: r_sP_c = 0.94, k = 3, P = 0.01) but not with rainfall (OH test: r_sP_c = - 0.94, k = 3, P = 0.99) or temperature regime (OH test: r_sP_c = - 0.47, k = 3, $P >$ 0.8) (Table 2). Among-populations differences in the proportions of branched hydrocarbons were marginally significant (GLM: population: Wald χ^2 = 5.618, *df* = 2, P = 0.060; Null deviance: 86.204 on 28 *df*, Residual deviance: 80.609 on 26 *df*) (Fig. 2C). Foundresses from Montgenèvre had marginally higher proportions of branched hydrocarbons than foundresses from Carì (Tukey's test, P = 0.060).

2.2 Multivariate dispersion of hydrocarbon profiles within populations

The within-population multivariate dispersions of the whole chemical profiles were significantly different (999 permutations, $F_{2,26}$ = 5.0236, P = 0.01), indicating that populations differed in the degree of among-colony variation in hydrocarbon profiles. The average distance to centroid for the three populations was lower in Carì (0.1120), than in Ferrere (0.1472) or Montgenèvre (0.1378) (Fig. 2D). Pairwise comparisons of group mean dispersion showed that Carì (where parasites were absent) differed significantly from both Ferrere (999 permutations, P = 0.004) and Montgenèvre (999 permutations, P = 0.042), whereas these two parasitized populations were not significantly between each other (999 permutations, P = 0.492) (Fig. 2D). The difference

Table 2 Mean concentration of hydrocarbons (± *SD*), proportion of branched hydrocarbons, parasite prevalence, mean daily maximum air temperature (± *SD*) and mean rainfall (±*SD*) in the three population (and relative population ranking).

Population	Hydrocarbon concentration (ng/mg of wasp)	Hydrocarbon concentration (rank)	Proportion of branched hydrocarbons (range)	Proportion of branched hydrocarbons (rank)	Parasite prevalence*	Parasite prevalence (rank)
Montgenèvre	317.88 ± 33.43	1	0.56 (0.48–0.63)	1	23.5%	1
Ferrere	223.60 ± 47.10	2	0.53 (0.41–0.60)	2	5.6%	2
Carì	219.90 ± 52.97	3	0.52 (0.47–0.58)	3	0%	3

Population	Max Temperature (°C)	Temperature (rank)	Mean precipitation (rain mm/day)	Precipitation (rank)
Montgenèvre	18.1±4.0	2	1.4±3.1	3
Ferrere	19.5±2.9	1	2.5±5.0	2
Carì	16.5±3.7	3	2.7±9.9	1

*measured as percentage of nests parasitized by *P. atrimandibularis* in 3-5 years, Lorenzi and Thompson, 2011.

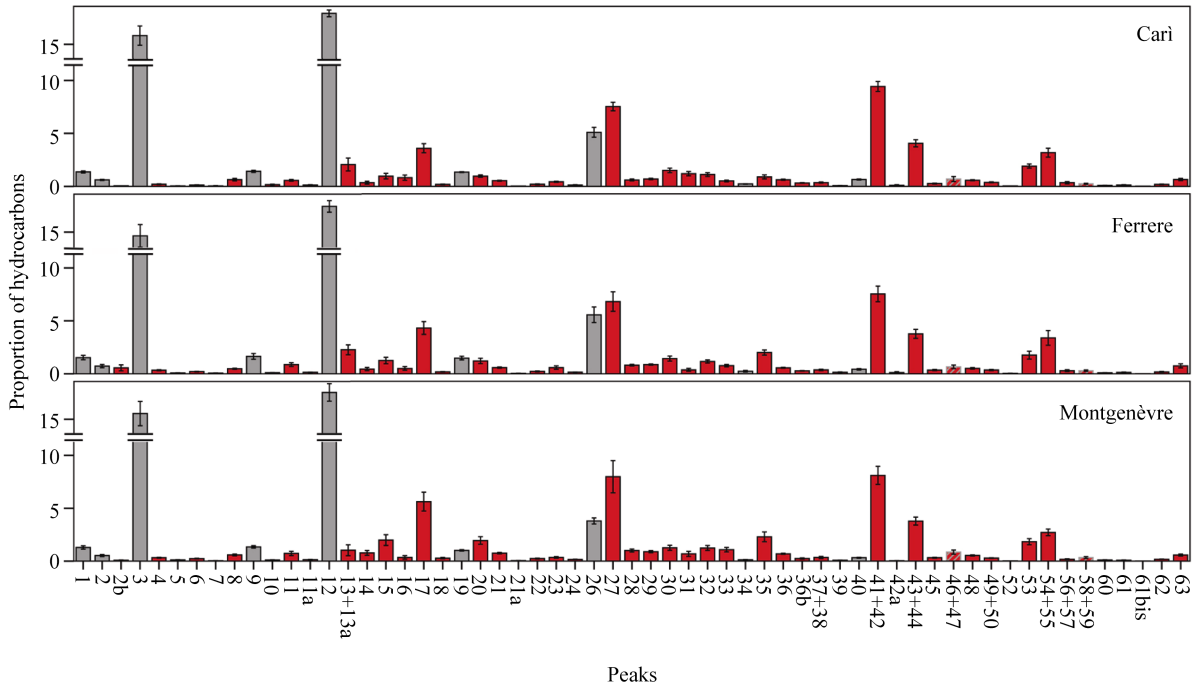

Fig. 1 The chemical signature of *Polistes biglumis* foundresses in the three populations (Carì, Ferrere, Montgenèvre)
The graph shows the average proportion of each peak (± *SE*) (linear alkanes: grey; branched alkanes: red; mixed peaks (peak 46+47 and 58+59): red-grey). Peaks are as follows: peak 1: *n*-C23; peak 2: *n*-C24; peak 2b: 2-Me C24; peak 3: *n*-C25; peak 4: 11-+13-MeC25; peak 5: 7-MeC25; peak 6: 5-MeC25; peak7: 11,15-DiMeC25; peak 8: 3-MeC25; peak 9: *n*-C26; peak 10: 12-MeC26; peak 11: 2-MeC26; peak 11a: 3-MeC26; peak 12: *n*-C27; peak 13+13a: mix of monoMeC27; peak14: 7-MeC27; peak 15: 5-MeC27; peak 16: 11,15-diMeC27; peak 17: 3-MeC27; peak 18: 5,11-+5,13-DiMeC27; peak 19: *n*-C28; peak 20: mix of diMeC27; peak 21: mix of monoMeC28; peak 21a: 6-MeC28; peak 22: 4-MeC28; peak 23: 2-MeC28; peak 24: 3-MeC28; peak 26: *n*-C29; peak 27: mix of monoMeC29; peak 28: 7-MeC29; peak 29: 5-MeC29; peak 30: mix of diMeC29; peak 31: 3-MeC29; peak 32: 5,11-+ 5,13-diMeC29; peak 33: 5,9-diMeC29; peak 34: *n*-C30; peak 35: mix of diMeC29; peak 36: mix of monoMeC30; peak 36b: 8,14-diMeC30; peak 37+38: 2-MeC30+9,19diMeC30; peak 39: 4,12-diMeC30; peak 40: *n*-C31; peak 41+42: mix of monoMeC31+7-MeC31; peak 42a: 5-MeC31; peak 43+44: 11,15-diMeC31+ mix of diMeC31; peak 45: 5,11- + 5,13-diMeC31; peak 46+47: *n*-C32+mix of diMeC31; peak 48: mix of monoMeC32; peak 49+50: mix of diMeC32 + 2-MeC32; peak 52: *n*-C33; peak 53: mix of monoMeC33; peak 54+55: mix of diMeC33; peak 56: mix of triMeC33; peak 58+59: *n*-C34+ mix of diMeC33; peak 60: mix of monoMeC34; peak 61: 2-MeC34; peak 61bis: *n*-C35; peak 62: mix of monoMeC35; peak 63: mix of diMeC35.

in within-population dispersion in chemical profiles of foundresses from the three populations is also visualized via NMDS (Fig. 3), where 95% ellipses are larger in parasite-infested populations (Montgenèvre and Ferrere) than in the parasite-free population (Carì).

3 Discussion

These results give empirical support for the hypothesis that social parasites impose directional selection on host hydrocarbon signatures and promote their variation. Mimetic social parasites selected hosts for increasing the concentration of cuticular hydrocarbons, the proportions of branched hydrocarbons and the within-population variation in chemical profiles. These three recognition signature traits had the largest values in the two parasitized population and the smallest values in the population where parasites were absent. They were therefore significantly and positively associated with parasite prevalence, suggesting that parasite pressure, and parasite chemical mimicry in particular, may act as a selective force that enhances the complexity of recognition codes and promote their communicative role.

The composition of cuticular hydrocarbon layers often reflects local climatic differences (Toolson, 1982; Gibbs et al., 1991; Wagner et al., 2001; Noorman and Den Otter, 2002; Parkash et al., 2008). Many insect species, though, have many internally-branched and unsaturated hydrocarbons on their cuticles. These hydrocarbons, owing to their high molecular complexity, have a high potential to encode information (LeConte and Hefetz, 2008; Blomquist and Bagnères, 2010; Richard and Hunt, 2013) and indeed qualify as effective communication agents (Dani et al., 2005; Lorenzi et al., 2011). The impact of abiotic factors (such as air temperature and rainfall regimes) on the cuticular blends of *P. biglumis* social wasps is not to be ruled out, but the patterns of hydrocarbon divergence between populations highlighted in this study are best explained by parasite prevalence rather than by local temperature or rainfall regimes. Abiotic factors were also poor predic-

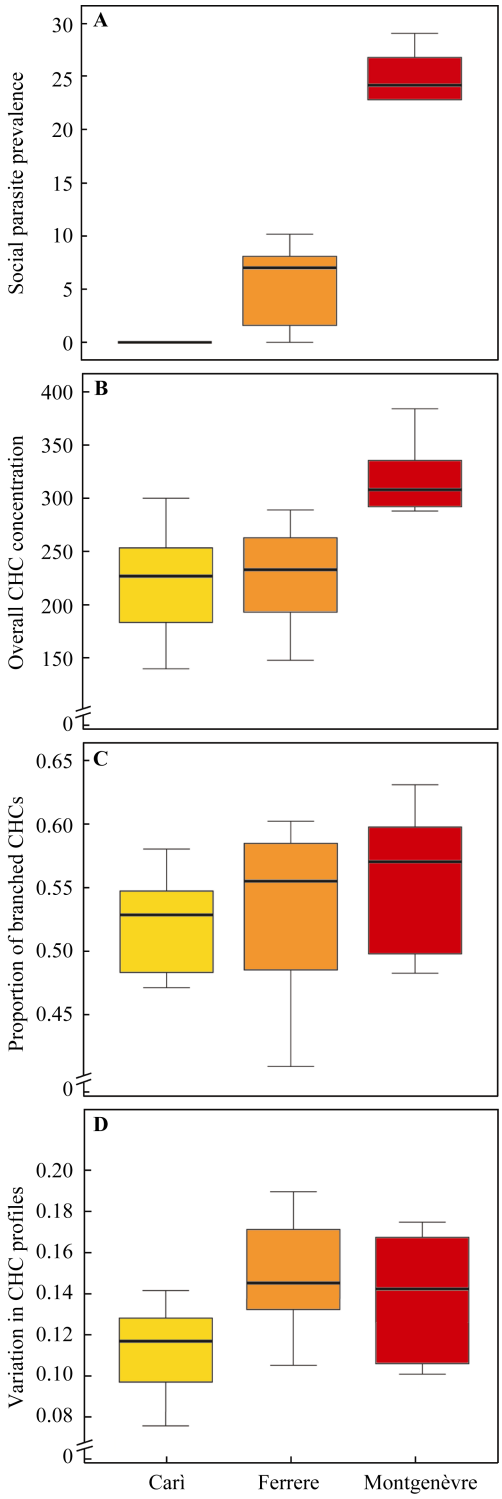

Fig. 2 Variation in parasite prevalence and chemical signatures among populations of *Polistes biglumis*

Variation is measured in terms of **A**) parasite prevalence (% of colonies parasitized by congeneric obligate social parasites); **B**) overall concentration of hydrocarbons (CHC) (in ng/mg of wasp); **C**) proportion of branched hydrocarbons (to branched + linear hydrocarbons) and **D**) quantitative variation in the chemical profiles (as within-population distance to centroid in a multidimensional Euclidean space). Carì: yellow; Ferrere: orange; Montgenèvre: red.

tors of the evolution of hydrocarbon signature in ants in phylogenetic analysis and comparative studies (van Wilgenburg et al., 2011; Menzel and Schmitt, 2011; Martin et al., 2013).

Our results also suggest that social parasites may promote an overall increase in cuticular hydrocarbon concentration in hosts. Generally, we do not know whether social parasites attain chemical mimicry through passive acquisition and/or biosynthesis of new compounds (Bagnères and Lorenzi, 2010; Bauer et al., 2010). However, producing and/or acquiring hydrocarbons - and branched hydrocarbons in particular - is costly (LeConte and Hefetz, 2008), leading to tradeoffs (Stearns, 1989; Roff and Fairbairn, 2007) in the expression of cuticular hydrocarbons. If social parasites do not exhibit enough of the appropriate hydrocarbons, they may fail to gain the membership status within host colonies (Lenoir et al., 2001; Lorenzi and Bagnères, 2010; Uboni et al., 2012). The need for parasites to gain membership may have selected for hosts that raise their concentration of recognition cues, advertising their identity with stronger cues, somehow exaggerating the concentration of cuticular hydrocarbons and especially their branched-hydrocarbon component. This, in turn, inadvertently raises the costs for co-evolving parasites: parasites that do not have enough recognition cues may have hard times in exploiting their hosts, as they may

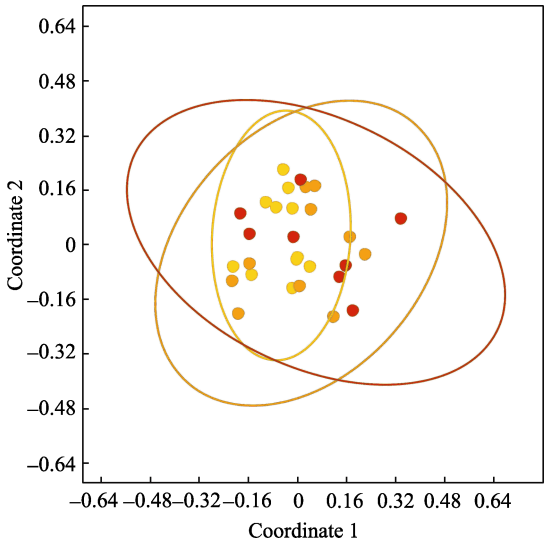

Fig. 3 NMDS plot of the chemical signatures of *Polistes biglumis*

Each dot represents the signature of a foundress from one of three populations (Carì: yellow; Ferrere: orange; Montgenèvre: red). The 95% confidence ellipses visualize the difference in multivariate dispersion of chemical signatures, which was larger in the two parasitized populations (Ferrere and Montgenèvre) than where parasites were absent (Carì).

be "invisible" to their host workforce.

We also analyzed the level of variation in recognition cues within and between populations. Bearers of rare signatures have higher chances of being rejected (and incurring fitness costs) than bearers of common ones: this would lead to erosion of signature diversity and to the eventual lack of a level of label polymorphism adequate to maintain recognition (Crozier's paradox) (Crozier, 1986; Tsutsui, 2004). In this scenario, it is unclear how polymorphism of recognition cues is maintained. One possibility is that colonies with rare signatures discriminate intruders better than those with common signatures (Ratnieks, 1991). Recently, a possible solution of Crozier's paradox has been suggested, involving the dual role of recognition cue in favoring both assortative cooperation and disassortative mating (Holman et al., 2013). Finally, recognition cue polymorphism might be maintained by selection imposed by parasites against common recognition cues (Martin et al., 2011).

Our results also suggest that parasites may promote the variation in the cuticular hydrocarbon profiles of their hosts. A larger cuticular hydrocarbon variation can be achieved qualitatively, by increasing the number of compounds in the cuticular blends, or quantitatively, by varying the relative proportions of compounds. For example, parasitized populations of *Formica fusca* ants had more dimethylalkane isomers than those that were parasite-free (Martin et al., 2011). Among *Camponotus* ants, the appearance of unusually long-chain unsaturated hydrocarbons might have evolved as a private communication channel within species (Menzel and Schmitt, 2011), possibly later used in the heterospecific context (Emery and Tsutsui, 2013). In our study, instead, we focused on quantitative variation in chemical profiles and found that the within-population variation in hydrocarbon proportions was associated with parasite prevalence. It could be argued that the lower level of within-population variation in the chemical profiles in Carì could be due to past bottlenecks through which this population might have passed. While it is true that we lack information to infer the demographic history of these populations, it is also true that past bottlenecks would explain the lower within-population variation in the chemical profiles in Carì, but would not make sense of the other trait values that we measured in the chemical signatures of the foundresses in this population. Instead, the relationship between within-population variation in hydrocarbon proportions and parasite prevalence may occur via negative, frequency-dependent selection, a form of balancing selection that promotes and maintains diversity (Zimmer and Emlen, 2013). Parasites may mimic hosts with common colony signatures more easily than those with rare colony signatures, in as much the same way as parasites infect common genotypes more easily than rare ones (Lively and Dybdahl, 2000; Kerstes et al., 2012). For example, hosts of avian brood parasites have evolved colour polymorphism in their eggs (i.e., between clutches in the same population) as an evolutionary response to egg mimicry by brood parasite pressure (Kilner and Langmore, 2011; Spottiswoode and Stevens, 2011). Similarly, our data suggest that host populations infected by social parasites had larger hydrocarbon-signature polymorphism than the parasite-free population, possibly as an evolutionary response to chemical mimicry by social parasites. It is suggestive that a previous study found that, in Ferrere and Montgenèvre, the two populations showing the largest chemical signature polymorphism, foundresses mate with multiple males, unlike most *Polistes* wasps (Seppä et al., 2011). Multiple mating has been often explained as an evolutionary response to parasite pressure (Sherman et al., 1988; King and Lively, 2012). Multiple mating by *P. biglumis* foundresses might enhance brood genetic heterogeneity, including chemical-signature heterogeneity. Indeed, selection by parasites may favour host foundresses with unusual proportions of hydrocarbons, as such foundresses are temporarily "out of reach" of coevolving social parasites. We know that social parasites exhibit host choice by preferentially targeting large colonies (e.g., Nash et al., 2008; Lorenzi and Thompson, 2011) and less aggressive hosts (Foitzik et al., 2001). It seems reasonable that they can also select hosts on the basis of their colony signature, since signature has a key role in parasite adaptation.

Nestmate recognition codes show remarkable chemical complexity, involving multiple biochemical pathways (Tsutsui, 2013). This complexity provides the opportunity to evaluate the ecological and social conditions that favor the evolution of complex signaling in relation to parasite pressure through comparisons within species or between host and non-host species. It also provides an example of how parasites may act as sources of selection which promote the diversification of communication signal between populations, eventually leading to a geographic mosaic of recognition codes (Thompson, 2005).

This is to our knowledge the first evidence in favour of the hypothesis that different trait in the host hydrocarbon signatures as a whole undergo evolutionary

changes resulting from either directional or balancing selection imposed by social parasites. While the number of populations sampled is quite low, this is a very promising finding, which will hopefully be confirmed by the sampling of additional populations.

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