

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B

BIOLOGICAL SCIENCES

Rethinking recognition: social context in adult life rather than early experience shapes recognition in a social wasp

Journal:	<i>Philosophical Transactions B</i>
Manuscript ID	RSTB-2019-0468.R1
Article Type:	Research
Date Submitted by the Author:	20-Feb-2020
Complete List of Authors:	Cappa, Federico; Università degli Studi di Firenze Cini, Alessandro; University College London Signorotti, Lisa; Università degli Studi di Firenze, Dipartimento di Biologia CERVO, Rita; Università degli Studi di Firenze, Biologia ;
Issue Code (this should have already been entered but please contact the Editorial Office if it is not present):	SIGNAL
Subject:	Behaviour < BIOLOGY
Keywords:	ontogeny of recognition, referent template, nestmate recognition, social insects, Polistes

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Wasps used in this work were reared under laboratory conditions and treated as well as possible given the constraints of the experimental design. This study was carried out in accordance with the Italian guidelines on animal wellness.

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Yes

Statement (if applicable):

The datasets supporting this article have been uploaded as part of the Supplementary Material.

Conflict of interest

I/We declare we have no competing interests

Statement (if applicable):

CUST_STATE_CONFLICT :No data available.

Authors' contributions

This paper has multiple authors and our individual contributions were as below

Statement (if applicable):

FC, RC and LS conceived and designed the study. FC and RC performed the experiments. AC carried out data analyses. RC provided materials and facilities. FC drafted the manuscript and all authors corrected, read and approved the final version of the manuscript.

Rethinking recognition: social context in adult life rather than early experience shapes recognition in a social wasp

Federico Cappa¹, Alessandro Cini^{1,2}, Lisa Signorotti¹, Rita Cervo¹

¹Dipartimento di Biologia, Università di Firenze, Firenze, Italy

²Centre for Biodiversity and Environment Research, University College London, London, United Kingdom

Abstract

Social recognition represents the foundation of social living. To what extent is social recognition hard-determined by early-life experience or flexible and influenced by social context of later life-stages is a crucial question in animal behaviour studies. Social insects have represented classic model to investigate the subject, and the acknowledged idea is that relevant information to create the referent template for nestmate recognition (NMR) is usually acquired during an early sensitive period in adult life. Experimental evidence, however, highlighted that also other processes may be at work in creating the template, and that such a template may be updated during adult life according to social requirements. However, currently we lack an ad hoc experiment testing the alternative hypotheses at the basis NMR ontogeny in social insects. Thus, to investigate the mechanisms underlying the ontogeny of NMR in *Polistes* wasps, a model genus in recognition studies, and their different role in determining recognition abilities, we subjected *Polistes dominula* workers to different olfactory experiences in different phases of their life before inserting them into the social environment of a novel colony and testing them in recognition bioassays. Our results show that workers develop their NMR abilities based on their social context rather than through preimaginal and early learning or self-referencing. Our study demonstrates, for the first time, that the social context represents the major component shaping recognition abilities in a social wasp, therefore shedding new light on the ontogeny of recognition in paper wasps and prompting the reader to rethink about the traditional knowledge at the basis of the recognition in social insects.

Keywords: ontogeny of recognition, referent template, nestmate recognition, social insects, *Polistes*

INTRODUCTION

Understanding the timing and cues of social recognition

Recognition represents the essential foundation of animal interactions. From sexual encounters between potential partners to the most complex animal societies, each individual has to recognize the ones encountered in order to decide how to behave towards them. Recognition occurs

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3 39 when specific phenotypic cues, or labels, expressed by an individual ('cue-bearer') are perceived by
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5 40 another individual ('evaluator') and then compared with an internal "referent template" of the latter
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8 41 [1-4]. According to the degree of correspondence between the encountered phenotype labels and the
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10 42 referent template, the evaluator performs the consequent recognition action, ranging from strong
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12 43 rejection to complete acceptance [1-5]. Given the spread and importance of recognition processes,
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14 44 the mechanisms at their basis have been extensively studied over the years both in vertebrates and
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17 45 invertebrates [2-8].

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19 46 As regards the nature of the cues potentially used by animals to create their template and
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21 47 compare the phenotype of others, different sensory channels are often involved depending on the
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24 48 species and the different recognition contexts [9-12]. Discrimination can occur through the use of
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26 49 environmental (e.g. nest odour) or genetic cues: animals can learn their own phenotypes (self-referent
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28 50 phenotype matching) or those of their relatives, and later compare the phenotypes of unknown
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30 51 individuals to this learned recognition template [2-5,7,8,13].

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33 52 Various processes explain when and how an animal acquires the crucial cues to create a
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35 53 template and perform correct phenotypic comparisons [2-5,14-17]. In particular, early experience
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37 54 coupled with a short sensitive period has been claimed to be a common and widespread evolutionary
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40 55 strategy to restrict the storage of biologically relevant information to a precocious developmental
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42 56 stage of the individual, thus reducing the risks of future recognition errors [15,17-21]. From the
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44
45 57 pioneer work of Konrad Lorenz [22] to the wide body of following research, early experience leading
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47 58 to imprinting-like phenomena appeared to play a major role in shaping recognition in animals [15,18-
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49 59 21,23-25]. Information acquired during an early sensitive window for learning are used by individuals
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51 60 across different contexts, to recognize parents and offspring [21,26,27], to discriminate kin from non-
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54 61 kin [28,29], to choose a sexual partner of the same species [24,30-33] and to select the most profitable
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56 62 food source [34-36]. In more recent years, however, a growing attention has also been devoted to the
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58 63 importance of the social surroundings of an animal, especially for those species living in socially
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60 64 structured groups, for the development of efficient recognition systems, with different studies

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3 65 highlighting that in impoverished social circumstances individuals struggle to develop adequate
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5 66 recognition patterns [19,37-41]. In both vertebrates [37-39] and invertebrates [40,41] social isolation
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7 67 affects or impairs the individual ability to recognize other group members.
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10 68 A social group is usually a highly complex environment, which can change over time both in
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12 69 the composition and social role of their members, and recognition processes appear to be finely
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14 70 entwined to the social network of interactions that an individual experiences through its life [42,43].
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16 71 Given the plasticity in the structure of many social environments [44-46], it may be evolutionary
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18 72 advantageous that group members should reflect a certain degree of plasticity in their recognition
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20 73 system with the ability to adjust or update their templates based on contextual contingencies
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22 74 [42,43,47]. For example, in many social insect species, the colony odour is dynamic and can change
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24 75 over time due to multiple factors (i.e. colony age, usurpation or replacement of the dominant
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26 76 individuals, colony relocation to a new nesting site, social interactions and cues exchanges among
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28 77 colony members, presence of parasites or pathogens, etc.) [48-56], therefore, for the recognition
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30 78 system to maintain its efficacy, at least in some species, individuals should be able to update their
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32 79 template during their life [43,47]. In fact, experimental evidence [57-59] suggests that the sensitive
33
34 80 early-life period might not be a shared feature in social species, opening the possibility that templates
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36 81 might be repeatedly updated during adult life according to social requirements [43,47,59,60]. Various
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38 82 species of ants showed a rather temporal flexible learning system for recognition cues [43,48,49,51]
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40 83 and also honeybees appear to readjust their recognition template after shifts in their colony odour
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42 84 [13,50,52].
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49 85 In animal societies, a type of recognition essential to direct altruistic efforts towards the
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51 86 appropriate targets, maintain the group integrity and defend it from outer threats or exploitation is
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53 87 represented by the discrimination of group members with respect to alien conspecifics [61-62].
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55 88 Nestmate recognition (NMR) represents a remarkable process especially in social insects' colonies,
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57 89 where is usually mediated by chemical cues (i.e. a blend of cuticular hydrocarbons, CHCs, covering
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59 90 the body surface of an individual) that are qualitatively similar in a species but can vary in their
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3 91 relative amounts among colonies of the same species [12,63]. In many social insects' species NMR
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5 92 is widely thought to be determined by early life experiences [43,47]. However, previous research has
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7 93 demonstrated that also other mechanisms can shape NMR abilities in social insects [43,51,57-61]
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10 94 and, in particular, recent findings on *Polistes* paper wasps might overturn our current understanding
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12 95 of template formation in social wasps [59,64]. These studies have demonstrated that also the social
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14 96 interactions and the environment experienced in adult life can alter the individual NMR
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17 97 [40,41,51,53,59], challenging the common perspective of the acquisition of a rather rigid recognition
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19 98 template during a strict sensitive window [14,65]. At present, however, we lack an integrative study
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21 99 assessing the relevance of the different mechanisms (i.e. pre-imaginal learning, early experience, self-
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24 100 referencing, adult social context) at the basis of NMR in shaping recognition abilities in social insects.
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28 102 *Paper-wasps as model for the ontogeny of recognition processes*

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31 103 Paper wasps of the genus *Polistes* have represented for decades a model to study the ontogeny
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33 104 of NMR in social insects [7,14,66]. The acknowledged idea is that *Polistes* wasps learn the olfactory
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35 105 recognition cues from the paper of their natal nest during a short sensitive window, namely the first
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37 106 few hours (48-72h) after emergence [7,14,66]. The nest material conveys the same chemicals of the
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40 107 colony inhabitants, providing information about colony membership [67], and it has therefore been
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42 108 considered as the primary source of cues, necessary and sufficient, for the acquisition of the referent
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44 109 template [67]. Although it was generally believed that the NMR was shared within the *Polistes* genus,
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47 110 the ontogeny of such a process has been investigated in only few of the 206 species of *Polistes* paper
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49 111 wasps and recent studies, carried out on the European paper-wasp *Polistes dominula* [47,59,64], have
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51 112 unveiled that the precocious sensitive window coupled with the nest paper as relevant cues do not
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54 113 represent the only mechanism to explain the development of recognition abilities in these wasps. In
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56 114 fact, these studies have already demonstrated that, at least in *P. dominula*, the template is not formed
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58 115 neither during an early post-emergence window [59] nor during the pupal stage [64] and that the nest
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3 116 paper is not the most relevant source of cues for template formation [59]. Such experimental evidence
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5 117 opened new questions on the mechanisms underlying the ontogeny of NMR in *Polistes* wasps.
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10 119 *Investigating recognition ontogeny through phase-dependent manipulation of olfactory experience*
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12 120 To investigate the mechanisms underlying the ontogeny of NMR in *Polistes* wasps,
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14 121 individuals in a specific stage of development can be subjected to differential odour experience
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16 122 experiments and, subsequently, to different social contexts, by introducing post-sensitive window
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18 123 adults into a novel social environment (i.e. nest). Individuals can then be tested in recognition
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20 124 bioassays recording the behavioural response (aggressive acts) towards different wasp lures (i.e.
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22 125 wasps freshly killed by freezing) (Fig.1). Such an experimental design allows to identify the precise
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24 126 timing and the most relevant cues for template formation. For example, if the referent template is
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26 127 acquired due to olfactory experience during an early sensitive window post emergence, it is possible
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28 128 to predict that, once tested in recognition bioassays, wasps will accept (i.e. lower aggressive response)
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30 129 lures bearing odour cues similar to those experienced by the wasp during the sensitive phase (Fig.1*
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32 130 This protocol was adopted by Signorotti and colleagues [59], which removed pre-eclosing workers
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34 131 and pupae of *P. dominula* from their natal nests and exposed them to different odour experience
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36 132 (odour cues from natal/foreign nest, no odour cues) during an alleged sensitive window (pupal stage
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38 133 or first hours post-emergence)[14] before reintroducing them into their colony of origin. Once tested
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40 134 in recognition bioassays wasps showed a higher acceptance of lures from their natal nests, regardless
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42 135 of their early olfactory experience [59]. Based on these results we can hypothesize that the referent
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44 136 template of the tested wasps was acquired either before the pupal or early adult sensitive window, i.e.
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46 137 during the larval stage on the natal nest, or after, i.e. once reintroduced into their natal colony (Fig.
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48 138 1), or through a different process of self-referent learning. Here, through a similar experiment of
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50 139 manipulation of early odour experience and of the social environment of adult wasps (introduction
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52 140 into a foster nest), we further investigate the mechanisms responsible for template formation in the
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54 141 same species, to finally assess the different importance of self-referencing, pre-imaginal or early
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3 142 olfactory experience, and social context in shaping the wasps' recognition abilities. In particular, our
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5 143 experimental design (Fig.1) allows us to predict that 1) if the referent template is acquired during the
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8 144 larval stage or it is based on a self-referencing process, wasps will be less aggressive towards nestmate
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10 145 lures from their natal nest with respect to wasp lures from the foster colony or from an alien colony;
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12 146 2) if the template is instead updated due to the olfactory and social experience during adult life in a
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15 147 biologically relevant context such as the foster nest, wasps should be less aggressive towards foster
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17 148 nestmate lures than towards wasp lures from their natal nest or from an alien colony; 3) finally, if all
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19 149 the different processes (preimaginal larval experience, self-referencing and template update due to
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22 150 social context) play a role in shaping recognition abilities, experimental wasps should be less
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24 151 aggressive toward both the foster nestmate and natal nestmate lures while attacking the alien wasp
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26 152 lure never encountered before.

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29 153 The main aim of the present study is to identify the differential contribution of the processes
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31 154 involved in template formation in *Polistes dominula* to challenge the traditional view on the ontogeny
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33 155 of NMR abilities in social wasps.

34 35 156 36 37 157 METHODS

38 39 158 *Collection and Laboratory Rearing*

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42 159 Colonies of *P. dominula* (n=60) in which the first generation of workers had already eclosed
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44 160 were collected in late June 2015 in 3 different localities, at least 9 kilometres apart to avoid any
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46 161 relatedness among tested wasps, throughout Tuscany (Italy). All nests were in a comparable stage of
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49 162 development with approximately 80 cells and contained immature brood (i.e. eggs, larvae and pupae).
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51 163 Colonies were transferred in glass boxes (15×15×15 cm) and provided with sugar, fly maggots and
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53 164 water *ad libitum*. Boxes were kept in the laboratory under natural photoperiod at ~25°C. Wasps on
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56 165 the nests at collection were identified as foundresses or workers [68] and marked on the wings with
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58 166 different acrylic colours (Testor Enamel) to distinguish among them and from newly-emerged
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60 167 workers. After four days from collection, when marked adults were at least 3 days old, a time window

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3 168 essential to allow the development of a complete cuticular chemical profile in this wasps [69], five
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5 169 marked workers were removed from each nest and killed by freezing to be used later as lures.

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8 170 To identify and remove pre-eclosing wasps from their natal nest at the end of their pupal stage,
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10 171 to ensure that they were not exposed to their colony odour or social environment in the early phase
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12 172 of their adult life, we used the same protocol adopted by Signorotti et al. [59]. Cell caps were partially
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14 173 removed before wasps' emergence with clean forceps and the colour and movements of pre-eclosing
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16 174 workers were observed. Developing pupae with both bright yellow/black colours and moving heads
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18 175 and antennae were selected as pre-eclosing workers according to [59].
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23 24 177 *Experimental Design*

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26 178 Pre-eclosing workers were gently removed from their cells with soft tweezers and individually
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28 179 transferred into plastic Petri dishes (2×1.5cm). Workers were divided into three groups, which
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30 180 experienced a different odour exposure during the early hours of their adult life: 1) 21 wasps
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32 181 (“C”=Control) were transferred to Petri dishes containing natal nest material (corresponding to about
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34 182 three empty cells)(odour cues) and a piece of filter paper (2×1cm); 2) 21 wasps (“F”=Familiarized)
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36 183 were transferred to Petri dishes containing a comparable amount of nest material (odour cues) from
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38 184 a foreign nest of a different population and a piece of filter paper (2×1cm); 3) 22 wasps (“N”=Neutral)
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40 185 were transferred to Petri dishes containing a piece of filter paper (2.5 cm²) to control for the effect of
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42 186 the paper material without odour cues. For all treatments, filter paper was previously washed with
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44 187 pentane for 15 minutes to remove any contaminations. Thus, the odour cues were present exclusively
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46 188 on the nest paper inside the Petri dishes in treatments “C” and “F”. Washed filter paper was added as
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48 189 a neutral control of the paper material and to have a similar amount of paper in the three different
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50 190 treatments. Petri dishes were provided with a hole for air and a small bee-candy (68% sucrose, 32%
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52 191 glucose-fructose syrup) as food for the wasp. To ensure that the sensitive phase for learning (few
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54 192 hours after emergence) reported for other *Polistes* species [7,66] was included in our experimental
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56 193 temporal window, wasps were left in Petri dishes for four days. To investigate the effect of the social
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3 194 contexts after the early olfactory experience, the post-sensitive window adult wasps were introduced
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5 195 into a novel social environment (i.e. foster nest). Each wasp was then individually marked and
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8 196 transferred to a foreign nest (foster nest) from a different population before being tested in recognition
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10 197 bioassays recording the behavioural response (aggressive acts) towards different wasp lures the
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12 198 following day, to allow acclimatization [59]. “F” workers were transferred to the nests that had
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15 199 provided the nest fragments for the early exposure into Petri dishes. Nests were maintained at 8°C in
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17 200 a refrigerator for 1h before introducing the focal wasp in order to calm down the resident wasps and
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19 201 facilitate acceptance of focal wasps by foster nestmates. Nests were monitored for 1 hour after
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21 202 introduction of focal wasps to evaluate the behavioural response of resident wasps towards the foreign
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24 203 individual. Regardless of treatment, focal wasps approaching nests were inspected by resident wasps
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26 204 with their antennae, but no particular aggressive reaction was recorded towards the introduced
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28 205 individuals. After acceptance, experimental wasps were left for 24h on their foster colonies before
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31 206 performing recognition bioassays. Signorotti et al. [59] already proved that a time window of 24h on
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33 207 a nest is sufficient for the focal wasps to develop their NMR abilities and and thus to perform the
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35 208 typical behavioural repertoire towards approaching intruders (presented lures) [70-72]. Another four
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38 209 wasps belonging to the three different treatments (1 “C”, 1 “F”, 2 “N”) did not approach the nest
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40 210 during the hour post introduction into the cage and were not on the nest the following day, thus, they
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42 211 were not tested in recognition bioassays.
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46 47 213 *Recognition Bioassays*

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49 214 Before starting the bioassays, all resident wasps were removed from each nest except the
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51 215 experimental individual to avoid any interference from resident wasps [59]. Each experimental wasp
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54 216 was left undisturbed on its nest for at least 15 min. A total of 60 experimental wasps (20 “C”, 20 “N”
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56 217 and 20 “F”) were tested. To evaluate the recognition abilities of experimental individuals, we
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58 218 presented each wasp with three different kinds of lures and we recorded their behavioural responses
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60 219 [34]. Lures were represented by a worker from the natal nest, a worker from the foster nest and an

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3 220 alien worker from a foreign colony of a different population. Lures were thawed to room temperature
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5 221 before trials. During bioassays, each lure was held with forceps and slowly introduced into the cage
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8 222 containing the experimental wasp on its adoptive nest. The lure was held about 1 cm from the nest
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10 223 and maintained for 1 min after the first contact between the focal wasp and the lure (bite or antennal
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12 224 inspection). Aggression toward a lure could depend in part on how threatening the lure is perceived
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15 225 to be because of both its size and appearance. Each triplet of lures was thus created using wasps that
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17 226 had comparable dimensions, measured as maximum head width under a Wild M5A stereomicroscope
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19 227 (difference in head width among lures of each triplet was less than 1.17%) [73]. All the lures were
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22 228 mounted on the forceps with the same posture, by holding the lure legs with the forceps. The antennae
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24 229 of the lures were open wide in the same position with the help of forceps and the stick bearing each
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26 230 lure was introduced into the cage containing the nest from the side opposite to the nest and slowly
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29 231 brought near the nest so that the focal wasp would see the face of the approaching lure. The three
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31 232 lures were presented to experimental wasps in a random order with a 30-min interval between
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33 233 presentations. Each lure was used only once. Experimenters performing and video-recording lure
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35 234 presentations were blind to lures' identity. Videos were blind-watched by a third observer. The
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38 235 number of bites given to each lure by the focal wasp was counted for statistical analysis. Bites are the
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40 236 most evident and quantifiable aggressive behaviour performed by wasps during this kind of
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42 237 experiment and aggressiveness towards wasp lures represent a good proxy of NMR since alien
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45 238 intruders are bitterly attacked and repelled while nestmates are inspected through antennation and
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47 239 peacefully ignored [59,70,71]. The general level of aggression among the treatment groups might
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49 240 inform about the motivation of wasps to defend the colony depending on their different early olfactory
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52 241 experience. Furthermore, as we provided contrasting olfactory versus social cues, our experimental
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54 242 design also allows to infer to what degree NMR is impaired by contrasting information during
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56 243 individual ontogeny. In fact, the combination of different early olfactory experience with social
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58 244 environment may have a modulatory or synergic effect on the behavioural response of the wasps. For
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60 245 example, "C" wasps which experienced olfactory cues from their natal nest during the early adult

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3 246 sensitive phase and contrasting social cues from the foster nest might respond less aggressively to
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5 247 lures from both the natal and foster nests with respect to aliens. Similarly, “F” wasps which
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8 248 experienced concordant early olfactory and social cues from the foster nest could defend more
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10 249 strenuously their adoptive colony and be more aggressive towards aliens with respect to “C” and “N”
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12 250 wasps.

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15 251 Finally, to verify if the introduction into a novel social environment (foster nest), not bearing any
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17 252 cues from the natal colony of the tested wasps, might alter their behavioural response, for example
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19 253 by reducing the motivation of the wasp to defend a foreign unrelated nest, we compared the level of
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21 254 aggression towards wasp lures recorded in our experiment with the aggressive response showed by
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24 255 wasps in the previous work using a similar experimental design [59]. In the study by Signorotti et al.
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26 256 [34] wasps were subjected to the same protocol of early olfactory experience, with the only difference
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29 257 that they were reintroduced into their own natal nests instead of a foster nest. The comparisons of the
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31 258 results from the present study with those from [59] should highlight the focal wasps’ ability to
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33 259 recognize their true colony of origin after being introduced on a nest. If focal wasps recognize their
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35 260 colony of origin from the foster colony due to cues learned through self-referencing or during the
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38 261 larval stage, we could expect a different behavioural response in the two experiments both in term of
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40 262 intensity and differential treatment of the presented lures. In fact, wasps could more intensively
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42 263 defend their natal colony to which they are related, while being less motivated in the defence of the
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45 264 foster colony.

49 266 *Statistical analyses*

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52 267 We used GEE (generalized estimating equations) to assess the importance of lure category (natal nest,
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54 268 foster nest, alien), treatment category (control, neutral or familiarized) and their interaction on
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56 269 aggressive response (i.e. number of bites given to each lure), and we set colony of origin for each
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58 270 focal wasp as subject effect to control for non-indipendence of focal wasp from the same nests (range
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60 271 of number of cases per subject was from 3 to 18). We used Poisson distribution with log-link function,

and an independent working correlation matrix structure. To calculate effect size we computed Cohen's d as: $d = \frac{m_a - m_b}{sd}$ where m_a and m_b are the estimated marginal means of each category within the pairwise comparison, and sd is the pooled standard deviation, which was calculated as $sd = \sqrt{\frac{(N_a - 1) * (sda)^2 + (N_b - 1) * (sdb)^2}{N_a + N_b - 2}}$ where N_a , N_b , sda and sdb are respectively sample size and standard deviation of each category [74]. The interpretation of d is as follows: small effect: $d=0.2$, medium effect: $d=0.5$, large effect: $d=0.8$ [75]. Non-parametric Mann-Whitney test was used to compare the aggressive response (number of bites) towards lures from the natal nests, alien lures and lures from the colony in which the wasps were reintroduced in the two experiments. All the statistical analyses were carried out using IBM SPSS 25.0.

RESULTS

We found a significant effect of lure category (Wald $\chi^2=61.949$, $df=2$, $p<0.001$) and no effect of treatment category (Wald $\chi^2=0.238$, $df=2$, $p=0.888$) on the focal wasp aggressive response. Indeed, the same pattern of NMR was evident for the three treatments (no significant interaction between lure category and treatment category: Wald $\chi^2=3.079$, $df=4$, $p=0.545$): lures from the foster nests were less attacked than aliens and lures from the natal nests, which were instead attacked similarly among them (Table 1). On average, lures from the foster nests received approximately six times less bites than lures from the natal nests or aliens across the three treatments (respectively 6.18 ± 0.12 and 5.80 ± 1.59 , Fig.2). In all cases the effect size was similar ($d \sim 0.2$, Table 1) indicating that the pattern of NMR response (aggression) was similar across treatments.

As regards the comparison with the results from the previous study [59], focal wasps did not show a significant difference in the aggressive response towards alien lures in the two experiments (Mann-Whitney U test, $Z=1.693$, $p=0.091$)(Fig 3a), whereas they responded differently to the lures from the natal nests (Mann-Whitney U test, $Z=-6.298$, $p<0.0001$), which were fiercely attacked in our bioassays while accepted in the previous experiment (Fig. 3b)[59]. Most interestingly, lure from the

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297 foster nests of our experiment were treated as lures from the natal nests in the former one (Mann-
Whitney U test, $Z=-0.646$, $p=0.477$, Fig. 3c).

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DISCUSSION

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Our results unambiguously show that in the social paper-wasp *Polistes dominula* template formation or update occurs during adult life, regardless of self-referencing and previous pre-imaginal or early post-imaginal olfactory experiences and actual nestmateship.

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Experimental wasps responded to different lures as if the lure from the foster colony was recognized as nestmate while the lure from the natal nest and the alien lure were equally treated as foreign individuals and strenuously attacked to defend the adoptive nest. The wasps' inability to recognize lures from their actual colony of origin suggests that our workers did not rely either on self-referent, preimaginal or early olfactory cues [3,4,14], otherwise we could have expected a lower aggressive response towards lures from the natal nest [14, 59,61]. Such inability is unlikely due to lack or insufficiency of relevant cues, since workers had sufficient time to acquire olfactory cues in the alleged early sensitive period [7,14,66,69] before being introduced into their foster colony. Instead, focal wasps strenuously defended their adoptive nests against both natal nest and alien lures highlighting that they considered the foster colonies as their true colonies of origin. If workers were able to use self-referent cues or to discriminate those from their natal colony, we might have expected them to put little effort into defence of the foster nest against intruders, especially towards individuals from their colony of origin, but this does not seem the case for our wasps. Interestingly, the comparison of our results with those of the previous study by Signorotti et al. [59], highlighted a similar pattern of aggressive response of focal wasps towards alien wasp lures, which means that they were equally motivated to defend the nest in both the experimental contexts. Instead, the degree of aggressiveness towards lures from the natal nests greatly varied between the two experiments, as they were accepted by wasps in [59] and bitterly repelled, similarly to alien intruders, in the present experiment. Such a result, suggesting that focal wasps were unable to recognize nestmates from their

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3 323 actual colony of origin, becomes even more interesting when considering the fact that our lures from
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5 324 the foster colonies were treated as lures from the natal nest in [59], since both were accepted by focal
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8 325 wasps. The indiscriminate acceptance of lures from the colonies in which experimental wasps were
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10 326 introduced (natal nest in [59], foster colony in the present study) in both experiments suggests that
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12 327 workers recognized as nestmates the lures coming from the first nest that they encountered during
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15 328 adult life. It would be possible to argue that the major role of the social context represented by a nest
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17 329 with its inhabitants in shaping NMR abilities with respect to an early olfactory experience may be
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19 330 simply due to the different entity of the chemical stimuli represented by a fully developed colony
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22 331 versus a limited amount of nest paper. However, the previous literature on NMR in *Polistes* wasps
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24 332 [7,14,66], from which the model for the ontogeny of NMR recognition in social wasps has been
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26 333 drawn, demonstrated that *Polistes* wasps belonging to different species were able to acquire the
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29 334 necessary cues for developing a NMR template even after being exposed for a very short time (1 or
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31 335 2 hours) to nest fragments [66], while exposure to adult nestmates outside the social environment of
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33 336 the colony did not allow the development of correct NMR abilities [76]. Our results may appear in
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35 337 contrast with these first studies which have investigated the ontogeny of recognition abilities in
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38 338 *Polistes* wasps, demonstrating that exposition to the sole nest paper during the first few hours post-
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40 339 emergence was necessary and sufficient to form a reliable referent template [7,14,66,76,77].
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42 340 Nevertheless, previous research on the ontogeny of NMR was carried out using other temperate
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45 341 species of *Polistes* as model species [7,66,76,77] and it is possible that different species might evolve
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47 342 different strategies, involving also different sensory channels, to recognize potential intruders [72,78].
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49 343 Moreover, these studies [7,66], as well as most of the pioneering researches exploring the ontogeny
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52 344 of recognition abilities in both vertebrates and invertebrates, tested the existence of a critical window
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54 345 for cues-learning and template formation in experimental conditions of social deprivation [7,37-
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56 346 41,66]. Individuals were isolated for a period of time during different developmental phases,
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58 347 presented with specific cues and then tested in subsequent bioassays for their recognition abilities
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60 348 without being exposed to biologically relevant social milieux [7,37-41,66,77].

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3 349 The behavioural response showed by our *Polistes* workers looks similar to the behaviour of
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5 350 ant workers kidnapped by slave-making species. Slave-workers are kidnapped by slave-raiding ants
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8 351 at the pupal stage and eclose in the parasite nest, towards which they develop a social attachment
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10 352 joining in all aspects of colony labour [80]. It is generally thought for social insects that after eclosion
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12 353 individuals should imprint to the odor of their nest, adult nestmates and brood in any colony in which
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14 354 they eclose [80,81]. In most cases, this will be their natal colony, and all adult nestmates and brood
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17 355 will be more or less closely related. Thus, it should be adaptive for a worker to defend and take care
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19 356 of those individuals encountered in the nest where it ecloses [61,63,81]. Different species of social
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21 357 parasites of the genus *Polistes* appear to exploit such process by usurping host colonies and modifying
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24 358 their chemical profile during the pre-emergence phase before eclosion of the first generation of
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26 359 workers [82]. Social parasites benefit from this peculiarity since host workers eclosing from the
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28 360 raided brood or in the usurped colony imprint to the present social environment and are manipulated
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31 361 to work for individuals to which they are not related [80-82].
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33 362 Despite the potential exploitation from social parasites, updating the referent template in adult
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35 363 life may be beneficial for maintaining an effective NMR system since the odour of individuals, and
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37 364 consequently the odour of the colony they inhabit, can change over time [48,49,52,53]. This is
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40 365 especially true for those species of social Hymenoptera where colony members continually exchange
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42 366 recognition cues and need frequent social interactions with nestmates to maintain an accurate referent
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44 367 template reflecting the current shared odour of the colony, as demonstrated in several ant species
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47 368 [51,60]. Template updating has also been shown in the honeybee, *Apis mellifera*, where guard
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49 369 workers at the colony entrance change their referent template after a combs' transfer between hives,
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51 370 with an increase in acceptance of non-nestmates belonging to the 'comb donor' hive [52]. The transfer
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53 371 does not change the bees' individual odour, but guards update their colony odour template directly
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56 372 from the wax comb rather than from the contact with other conspecifics [52]. In social wasps no direct
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58 373 evidences for a template updating were available so far [47], but, once again, studies on social
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60 374 parasitism in *Polistes* wasps indirectly indicated a similar process of updating. For instance, *Polistes*

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biglumis workers recognize only the odour of their species in non-parasitized colonies, but learn a template that fits the odour of their own species and that of the social parasite *Polistes atrimandibularis* after colony usurpation [82]. Although exploited by social parasites, the ability to retard, adjust or update the referent template may still be advantageous in *Polistes* annual societies, where female functional caste is relatively labile and change at the top of the hierarchy, with subsequent variation of the colony odour, are likely, especially in those species, such as *P. dominula*, characterized by associative founding with several reproductive females, sometimes unrelated, cooperating to build a new colony at the beginning of the season but still competing over dominance [83,84].

Overall, our study demonstrates, for the first time in a social wasp, that the social context experienced in adult life can represent the major component for the formation of the NMR template, stronger than genetic cues of nestmateship or self-referencing and regardless of previous pre-imaginal or early post-imaginal olfactory experiences during time-restricted sensitive phases. We believe that our results prompt the reader to rethink about the traditional knowledge at the basis of the ontogeny of nestmate recognition in social insects, highlighting a previously unreported plasticity in the development of recognition abilities in insects, which are often considered as simple and stereotyped organisms, and opening new avenues for research.

Acknowledgements

The authors thank Dr. Daniele Venâncio for assistance while performing recognition assays and Prof. Patrizia d’Ettorre for fruitful discussion on the topics of early experience and nestmate recognition. Financial support was provided to RC from University of Florence.

Ethics

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3 399 Wasps used in this work were reared under laboratory conditions and treated as well as possible given
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5 400 the constraints of the experimental design. This study was carried out in accordance with the Italian
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8 401 guidelines on animal wellness.

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12 403 Data accessibility

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14 404 The datasets supporting this article have been uploaded as part of the Supplementary Material.

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19 406 Authors' contribution

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21 407 FC, RC and LS conceived and designed the study. FC and RC performed the experiments. AC carried
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24 408 out data analyses. RC provided materials and facilities. FC drafted the manuscript and all authors
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26 409 corrected, read and approved the final version of the manuscript.

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30 411 Competing Interests: The authors declare no competing interests.

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40 674 Figure 1. Experimental design used in the study. Wasps were subjected to different olfactory and
41 675 social experience in different phases of their life, from pre-imaginal (larval) to adult life and then
42 676 tested in recognition bioassays to assess which experience and phase was more relevant for the
43 677 ontogeny of NMR abilities. *The effect of early post-emergence olfactory experience (Prediction 2)
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45 679

46 680 Figure 2. Aggressive responses (number of bites) of experimental wasps towards wasp lures
47 681 belonging to the three different categories (natal nest; foster nest; alien) for the three experimental
48 682 treatments: a) "Control"; b) "Familiarized; c) "Neutral". Thick horizontal lines represent medians,
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50 684

51 685 Figure 3. Comparison of the aggressive responses (number of bites) of experimental wasps from the
52 686 present study and Signorotti et al. [59] towards alien lures (AL), lures from the natal nest (NT) and
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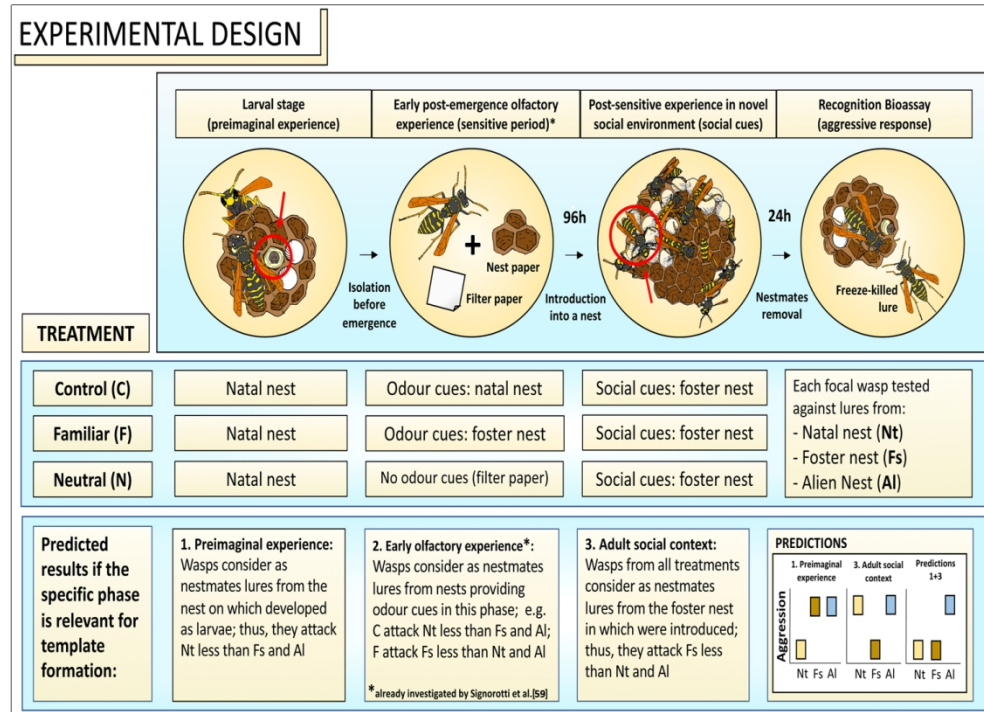


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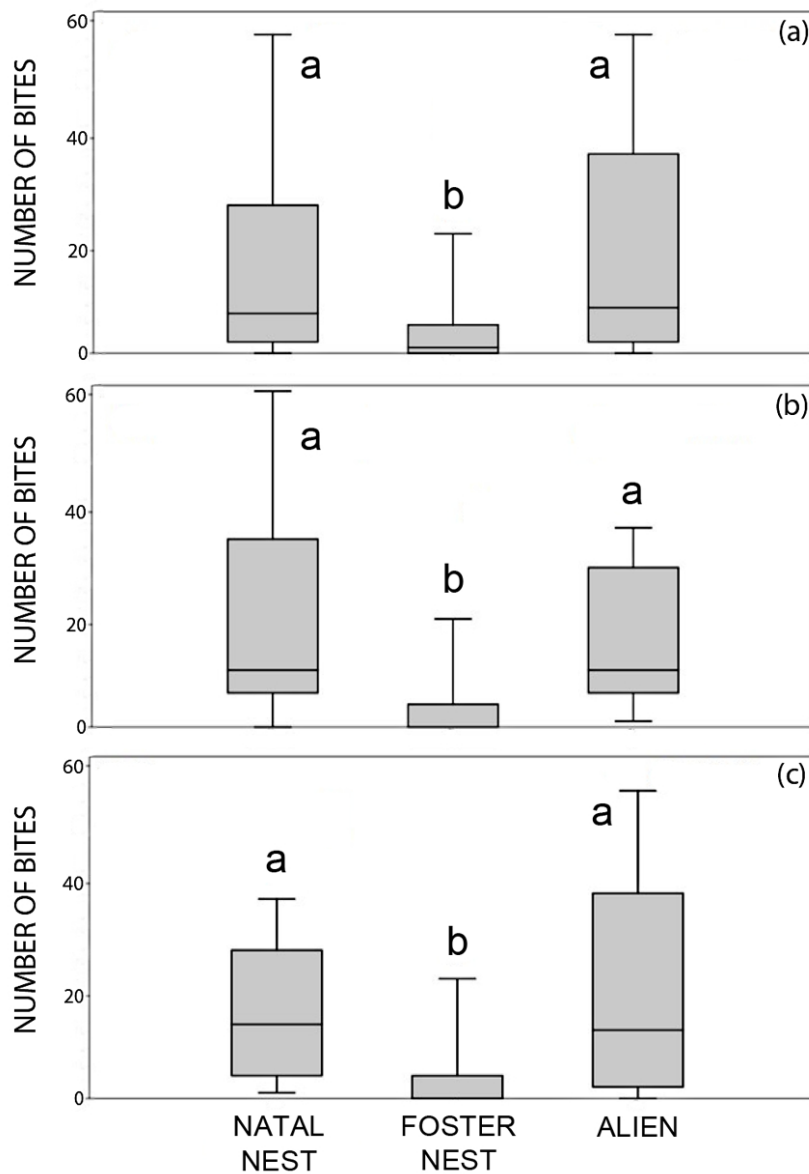


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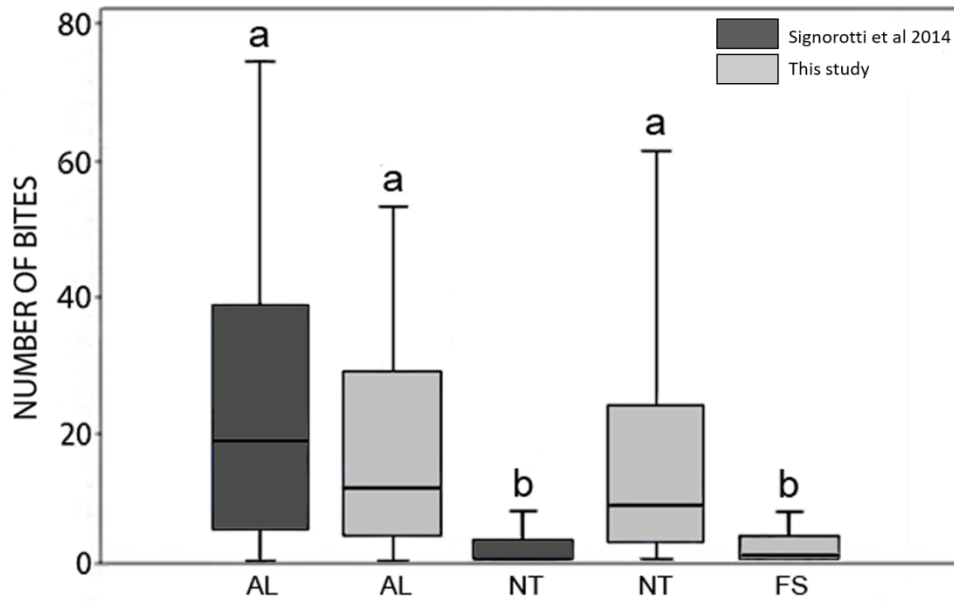


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3 1 Table 1. Results from GEE show that in all treatments focal wasps were less aggressive towards the
4 2 foster nest lure (Fs) rather than towards natal nest (Nt) and alien (Al) lures that were attacked at a
5 3 comparable rate. *aggression corresponds to number of bites received by each lure.
6 4

Treatment	Comparison	Average fold contrast in aggression*	Post-hoc stats	Effect size
Control	Nt vs Fs	4.800	0.033	0.190
	Alien vs Fs	6.182	0.001	0.139
	Alien vs Nt	1.288	0.996	na
Neutral	Nt vs Fs	4.968	<0.001	0.190
	Alien vs Fs	6.307	<0.001	0.203
	Alien vs Nt	1.270	0.771	na
Familiarized	Nt vs Fs	7.640	<0.001	0.277
	Alien vs Fs	6.060	<0.001	0.209
	Alien vs Nt	0.793	0.998	na