

Grooming Patterns in the Primitively Eusocial Wasp *Polistes dominulus*

A. Sumana & Philip T. Starks

Department of Biology, Tufts University, Medford, MA, USA

Abstract

Grooming is a commonly observed behavior in many animals. One function of grooming is to clean the body of debris and parasites. An additional function may be to homogenize chemical cues present on the body. This latter purpose is especially likely in species in which contact-based chemical communication occurs, such as in eusocial insects. In this study we address the context, sequence, frequency and duration of 683 acts of self-grooming performed by the paper wasp, *Polistes dominulus*. In general, individuals groomed heads after cell inspections, and abdomens after sitting, suggesting that grooming serves to remove debris from the body. Although no differences were observed in the total amount of time spent grooming, foundresses groomed significantly more often than did workers. Wasps were equally likely to groom thoraces or abdomens following heads, but were more likely to groom abdomens after thoraces and heads after abdomens. Interestingly, the appendages used to groom individual body parts were highly specific (e.g. the prothoracic legs were used for the head), thus indicating that grooming is not used to homogenize chemical cues across the body surface of the wasp.

Correspondence: Philip T. Starks, Department of Biology, Tufts University, Medford, MA 02155, USA. E-mail: philip.starks@tufts.edu

Introduction

Grooming is one of the most common behavior patterns of animals. Typically, grooming is classified as a function of both the performer and recipient of the behavior. When the two are the same, the behavior is called self-grooming, and when the two are different, the behavior is called allogrooming (Goodenough et al. 1993). In the present study, we consider self-grooming patterns of the primitively eusocial paper wasp *Polistes dominulus*.

Grooming has mostly been evaluated within the context of its functional significance (Jander 1976). Suggested functions include the removal of ectoparasites and the formation of coalitions (see Dugatkin 1997). Grooming in highly

social animals has implications not only for the individual but also for the group. Indeed, the increased rate of disease transmission facilitated by sharing a common site such as a burrow or a nest has often been considered one of the costs of social living (Alcock 2001). Although many studies of social insects have included grooming in activity profiles (Gadagkar & Joshi 1983; Reeve & Gamboa 1983; Theraulaz et al. 1989), investigations have not focused on grooming *per se*, instead considering it a part of an individual's behavioral repertoire.

Grooming to remove pathogens or parasites may only be one component of the adaptive significance of the behavior in social insects. Grooming may serve to homogenize chemical cues used in nestmate or caste recognition. Studies have shown that hydrocarbon profiles on a social insect's cuticle signify nestmate status (Howard 1993; Espelie et al. 1994; Blomquist et al. 1998; Lenoir et al. 1999; Pickett et al. 2000; Dani et al. 2001; Sledge et al. 2001a; Zanetti et al. 2001; Ruther et al. 2002) and indicate an individual's caste (Monnin et al. 1998; Singer et al. 1998; Peeters et al. 1999; Liebig et al. 2000; Cuvillier-Hot et al. 2001; Sledge et al. 2001b; Hannonen et al. 2002; Abdalla et al. 2003). The pathway involved in the synthesis of these cuticular hydrocarbons is not well-understood. In an attempt to understand this, researchers have examined the hydrocarbons present in various glandular secretions. In wasps and bees, similarities exist between the cuticular hydrocarbon composition and the hydrocarbons secreted by the Dufour's gland (an accessory gland of the female reproductive tract) (Dani et al. 1996, 2001). As cuticular hydrocarbons are relatively non-volatile, they would not be expected to automatically disperse over the entire body of an individual. Grooming has been considered a means through which these hydrocarbons are dispersed over the body (Ayasse et al. 2001).

Accordingly, we have two potentially complementary hypotheses for the function of grooming in paper wasps: the removal of debris (and/or pathogens and parasites) and the homogenization of cuticular hydrocarbons. While both hypotheses predict that grooming should be observed after contact with foreign substances, the homogenization hypothesis stands alone in requiring that appendages used to groom one body segment must also be used on other segments in order to spread the hydrocarbons. Without such cross-segment contact, individual body parts could be described as separate islands each with potentially different chemical signatures.

Body-wide distribution of chemical cues may be important for social interactions. Many, if not most, interactions involve only a specific part of the animal, rather than the whole body. One of the most common interactions is antennation – the contact of one individual's antennae to any body part of another individual. Antennations are of special importance because they are considered the means through which cuticular hydrocarbons are perceived (Clément 1982). It would be advantageous to have a homogenous chemical signature across the entire body, as this would permit a more uniform transfer of information, irrespective of which part of the body was contacted. Thus for any discussion of the production and perception of chemical cues in the context of recognition systems, it becomes essential to investigate the distribution of these

cues and how this distribution is achieved. As self-grooming can impact the spread of chemical cues and can be addressed through classical ethology techniques, it was the focus of this study.

Polistes dominulus is ideal for the investigation of grooming as recent research has indicated that both nestmate and caste status can be determined from its cuticular hydrocarbons (Lorenzi et al. 1997; Dani et al. 2001; Sledge et al. 2001b). We make a quantitative comparison between foundresses and workers in the frequency and duration of grooming. In addition, we study the context in which grooming is performed, the sequence in which the various body parts are groomed, and the appendages used for grooming.

Methods

Five pre-emergence colonies were collected in Massachusetts and were housed at the Tufts University ISIRF (International Social Insect Research Facility) in closed nest boxes ($60 \times 26 \times 22$ cm) with ad libitum water, honey-water, prey, and construction material. Colonies were each videotaped (Sony-Handycam vision TRV108) for 6 hr and all tapes were transcribed by one of us (AS). Colonies contained a total of 12 foundresses ($\bar{x} \pm SD$ per colony; 2.4 ± 1.1) and 17 workers (4 ± 3.1), all of which were individually marked. During the 30 hr of observation, we gathered information on 683 acts of grooming. For each act of grooming, we recorded (i) the behavioral state of the wasp just before she initiated grooming, (ii) the body part she groomed, (iii) the body structures she used for grooming, (iv) the duration she groomed, and (v) the behavior she performed immediately after the grooming event. In order to calculate the proportion of time spent in these activities, we also carried out scans every 6 min (30 scans per colony) and noted the behavioral states of the wasps.

Based on morphology, we classified grooming into three categories – grooming of the head, the thorax, and the abdomen. Each region was groomed in a characteristic manner. *Groom head*: a wasp would run her prothoracic legs (first pair) along her head and often pass the legs through her mouth. In addition, she would rub her prothoracic legs against her antenna, then pass her legs through her mouth and would conclude by brushing her prothoracic legs together. *Groom thorax*: a wasp would run her prothoracic legs (first pair) along her thorax, occasionally pass these legs through her mouth, and conclude by brushing her prothoracic legs together. *Groom abdomen*: a wasp would run her metathoracic legs (third pair) along her abdomen. In addition, she would, on occasion, rub her wings along her abdomen, and generally end the grooming by brushing her metathoracic legs together.

Comparisons of head, thorax and abdomen grooming were carried out using Wilcoxon signed rank tests. Comparisons between foundresses and workers both within and across grooming categories were carried out using Mann–Whitney U-tests. Chi-square tests were used to analyze the sequence of grooming, the body component groomed after a given behavior was performed, and the appendages used to groom a body section.

Results

We restricted our analysis of the pre-grooming behavioral states to sitting, walking, cell inspection and interaction with other wasps, because these represented 88.86% of all observed behaviors. Grooming accounted for 6.6% of the time activity budget, thus our analysis accounts for 95.14% of all non-grooming remaining behaviors. In no case could the body section groomed after a behavior be considered random (Table 1). In general, the thorax was groomed less often than expected under the assumption that all body segments are groomed equally. The most striking differences observed were between head and abdomen grooming: grooming of the head was significantly more common than expected after cell inspection (the insertion of the head into nest cells), and grooming of the abdomen significantly more common than expected after sitting.

Foundresses groomed more often than workers (foundresses 4.94 ± 2.14 ; workers 2.14 ± 1.66 freq/hr/wasp; Mann–Whitney U-test, $p < 0.05$), but both foundresses and workers spent equal time grooming themselves (foundresses 72 ± 2224.3 ; workers 66.94 ± 93.06 ; duration in seconds/wasp/grooming bout; Mann–Whitney U-test, $p > 0.05$). Foundresses groomed heads and thoraces significantly more often than did workers, while both groomed the abdomen at comparable rates (Fig. 1a). Foundresses and workers spent comparable time grooming their heads, thoraces and abdomens (Fig. 1b), and both spent more time grooming thoraces and abdomens than heads (Fig. 1b).

The order of body segment grooming observed in foundresses and workers was similar and thus we combined them for this analysis. After grooming the head, wasps were equally likely to groom either the thorax or abdomen (Fig. 2a). After grooming the thorax, however, wasps were significantly more likely to groom the abdomen than the head (Fig. 2b). As with the thorax, the segment groomed after the abdomen was not random: wasps were significantly more likely to groom the head after the abdomen (Fig. 2c).

Table 1: Observed and expected values for different behaviors observed immediately prior to grooming head, thorax and abdomen. All comparisons were carried out using chi-square tests. Expected values were calculated under the assumption that wasps initiate grooming at random and thus would have equal probability of grooming from any one of the behavioral states analyzed. None of the behaviors listed below were followed with a random pattern of grooming ($df = 2$, $p < 0.001$ for all comparisons). Individually significant values are in bold. ** $p < 0.001$

Behavior prior to grooming	Groom head		Groom thorax		Groom abdomen	
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
Cell inspection	104**	39	1**	39	12**	39
Sitting	83	73	6**	73	130**	73
Walking	63**	34.7	1**	34.7	40	34.7
Interaction	31**	13	0**	13	8	13

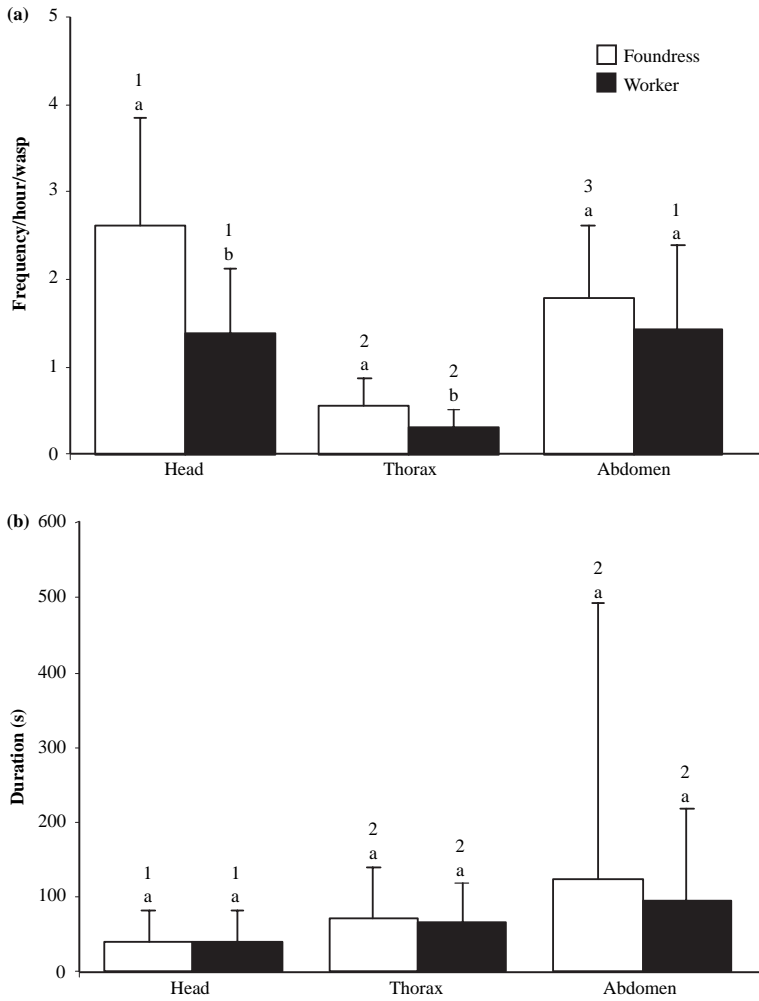


Fig. 1: (a) The frequency per hour per wasp, (b) the duration in seconds, at which grooming of the head, thorax and abdomen was performed by foundresses (white bars) and workers (black bars). Bars are mean and error bars represent standard deviation. All comparisons between foundresses ($n = 12$) and workers ($n = 17$) were by Mann–Whitney U-test and bars carrying different alphabets represent significantly different values ($p < 0.05$). All comparisons between head, thorax and abdomen within each category were carried out by Wilcoxon signed rank test, and bars carrying different numbers represent significantly different values ($p < 0.05$)

A stereotyped body segment grooming order could lead to a homogenization of cuticular cues across the body. For this to occur, however, the appendages used to groom each section of the body must not be specific to a given section. A detailed consideration of the appendages used for grooming showed that prothoracic legs were used to groom the head and thorax significantly more

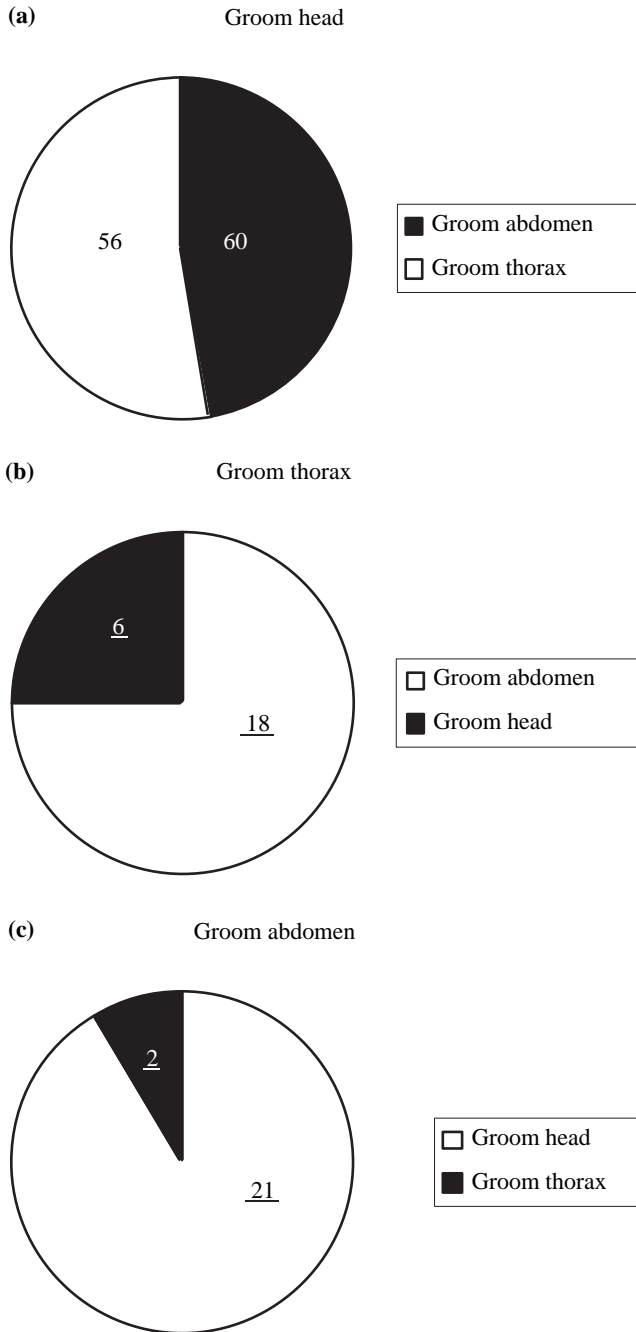


Fig. 2: Observed number of transitions recorded from grooming a focal area; head (a), thorax (b) and abdomen (c) to other parts of the body is represented in the pie diagram. The actual numbers are mentioned in each section of the pie chart and significantly different values are underlined (chi-square analysis, $p < 0.05$, $df = 1$)

Table 2: Observed values for the instruments used for grooming the head, thorax and abdomen, from three colonies and 420 acts of grooming. In no instance was the use of instruments random for a body part

	Head	Thorax	Abdomen	p (χ^2 , df = 2)
Prothoracic legs	203	50	2	<0.001
Mesothoracic legs	0	4	0	<0.02
Metathoracic legs	0	0	161	<0.001
Mouth	189	25	2	<0.001
Wings	0	2	42	<0.001

than were the mesothoracic or metathoracic legs, and the metathoracic legs were exclusively used to groom the abdomen (Table 2). As the abdomen is the segment most likely to contain Dufour's gland secretions, and as the use of different appendages for grooming seem specific to given body segments, these results suggest that grooming does not homogenize cues across an individual's body.

Discussion

Grooming is an extraordinarily common behavior observed across most animal species and is a common behavior performed by social insects. In spite of this – to the best of our knowledge – no detailed study of grooming in social wasps has been published. Here we examined two hypotheses for the function of self-grooming in wasps and conclude that, while the behavior may be used to remove debris, it does not appear that grooming is used to homogenize cuticular signatures across a wasp's body.

Our observations show differences between reproductive castes with respect to the number of grooming events, but not with respect to the amount of time spent grooming individual body parts (Fig. 1a, b). In general, wasps spent less time grooming the head than either the thorax or abdomen. In addition, the sequence of grooming was not random. Wasps were most likely to groom the abdomen after the thorax, and the head after the abdomen. No preference was detected for the thorax or abdomen after grooming the head (Fig. 2).

In agreement with a debris removal hypothesis, the head was groomed most often after cell inspection, while the abdomen was groomed most often after sitting (Table 1). In contrast to the cue homogenization hypothesis, appendages used to groom individual body segments were specific to those regions (Table 2): the head, antenna and thorax were groomed by the prothoracic legs, while the abdomen was exclusively groomed by the metathoracic legs. Thus it is possible for cues on the thorax to travel to the head or from the head to thorax, but cues on the abdomen do not appear to be spread by grooming.

These results suggest that grooming cannot be the means of homogenizing cuticular hydrocarbons across the body surface of the wasp. This finding raises questions regarding the similarity of the hydrocarbons present on the different parts of the wasps' body. Indeed, there data suggest that chemical cues on the

wasp's body are heterogeneous. In a behavioral investigation of the communication of status in *P. fuscatus*, the head and ovaries of the most dominant individual were recognized by the subordinates, while other parts of the dominant wasp were not recognized (Downing & Jeanne 1985).

Many recent studies have collected cuticular hydrocarbons from only one body part of the individual under study. For example cuticular hydrocarbons from just the head, the thorax, the wings or the abdomen was collected in order to correlate the cues with different behaviors and physiology (Monnin et al. 1998; Liebig et al. 2000; Sledge et al. 2001b; Abdalla et al. 2003). Our results, and those of Downing & Jeanne (1985), suggest that the head and thorax form one cue-island, and the abdomen forms another. As such, future research should compare the cuticular signatures within an individual. If chemical signatures are shown to differ between body sections, and if this difference influences the information content of the signal, a re-examination of previous cuticular hydrocarbon studies may be necessary.

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