



Research report

Asymmetry in antennal contacts during trophallaxis in ants

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ABSTRACT

Behavioural and brain left–right asymmetries are a common feature among the animal kingdom. Lateralization often manifests itself at the population-level with most individuals showing the same direction of lateral bias. Theoretical model based on evolutionary stable strategy predicts that lateralization at the population-level is more likely to characterize social rather than solitary species. Empirical data supporting this hypothesis has been recently obtained in Hymenoptera showing that eusocial honeybees present an asymmetrical use of the antennae: the right antenna is involved in olfactory learning and present more olfactory receptors. However, no evidences about the role of antennal asymmetries in social interactions have been provided so far. Highly social ant species belonging to *Formica rufa* group are a good model for investigating natural communication because they are able to pass exact information to their nest mates. We applied the “binary tree” experimental paradigm, which allowed us to observe different types of antennal contacts performed by ants out of their nest. To examine possible asymmetrical use of the right and left antenna, we focused on “feeding” (the simplest) contacts where a “donor” ant is exchanging food with a “receiver” ant through trophallaxis. We observed a population-level asymmetry, with the “receiver” ant using the right antenna significantly more often than the left antenna. This study provides the first evidence of lateralization in antennal contacts in ants, and seems to support the hypothesis of mathematical models on the evolution of lateralization suggesting that the alignment of lateralization at the population-level matters in social interactions.

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1. Introduction

Traditionally, only humans were thought to have strong left–right asymmetries in brain and behaviour. Now evidence for lateral biases affecting everyday behaviour of a variety of vertebrate species (reviews in [32,33]) is widespread and cerebral lateralization is certainly not a uniquely human attribute. Lateralized animals have been shown to outperform non-lateralized ones in many circumstances, suggesting that lateralization contributes significantly to biological fitness [11,16,26]. Recently, evidences about lateralization in invertebrates have started to emerge suggesting that lateralization of the nervous system may be a feature of simpler brains as well as more complex ones. A variety of studies have revealed several sensory and motor asymmetries in behaviour as well as asymmetries in the nervous system in invertebrates (reviewed in [8]).

Further, it has become apparent that two patterns of lateralization exist across species. In “individual-level” lateralization an equal number of left- and right-biased individuals coexist in the

species, while in “population-level” lateralization a majority of individuals is right- or left-biased. The latter is the case of humans, as exemplified by handedness, and of most of the non-human species studied so far. While individual-level lateralization may have evolved because it increases individual efficiency [1,15,24,30], population-level lateralization is unrelated to individual efficiency, and remained unexplained until a few years ago, when it was suggested that the alignment of lateralization at the population level may evolve as an evolutionary stable strategy (ESS) when individually asymmetrical organisms must coordinate their behaviour with that of other asymmetrical organisms [32]. Game-theoretical models developing this idea and considering group-living individuals engaging in intraspecific and interspecific interactions suggest that population-level lateralization is more likely to evolve in social than in non-social species [9,10].

Anfora et al. [2] tested this hypothesis empirically comparing olfactory lateralization in two species of Hymenoptera Apoidea, the honeybee (*Apis mellifera*), a social species, and the mason bee (*Osmia cornuta*), a solitary species. Recall of the olfactory memory 1 h after training to associate an odour with a sugar reward, as revealed by the bee extending its proboscis when presented with the trained odour (Proboscis Extension Reflex – PER – paradigm, [3]), was better in honeybees trained with their right than with

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their left antenna. No such asymmetry was observed in mason bees. Similarly, electroantennographic responses to a floral volatile compound and to an alarm pheromone component were higher in the right than in the left antenna in honeybees but not in mason bees. Results showed that both in behavioural (using the PER paradigm) and in electroantennography responsiveness, honeybees that are a social species are lateralized at the population level, while mason bees that are a solitary species are lateralized at the individual level. Very recently primitive social bees have been studied [7] to investigate the evolutionary origins of the asymmetry reported in honeybees [2,5,6,14,25]. Three species of Australian native, stingless bees (*Trigona carbonaria*, *Trigona hockingsi* and *Austroplebeia australis*) were trained to discriminate two odours using PER. Recall of the olfactory memory at 1 h after training was better when the odour was presented on the right than on the left side of the bee. In contrast, recall at 5 h after training was better when the odour was presented on the left than on the right side of the bee. Stingless bees show the same laterality as honeybees [25], suggesting that asymmetry evolved prior to the evolutionary divergence of these species. These findings [2,7] seem to support recent game-theoretical models suggesting that stable polymorphism with an uneven distribution of left- and right-forms can be expected to emerge spontaneously in species in which left–right biases have behavioural consequences during everyday interactions between individuals [9,10,31]. Note that olfactory learning and electroantennographic responsivity are not obviously social in nature although we cannot exclude that the original drive for antennal asymmetries could be related to social interaction during for example trophallaxis, i.e. the transfer of food or other fluids among members of a community. On the other hand, it is likely that when an individual-level asymmetry is stabilized as a directional population-level asymmetry, other asymmetries that in principle would not require any alignment at the population level because they are irrelevant to any social interaction would organize themselves as directional as well simply because a directional organization in the two sides of the brain already exists.

So far there is no evidence in honeybees about the role of antennal asymmetries in social interactions. Honeybees (*A. mellifera*) communicate through “dance language”: successful forager honeybees are able to recruit other bees to a distant goal by specific “dance” movements together with other components of communications such as odours and sounds [34]. Unfortunately, it is not so easy to investigate these interactions in a natural context because trophallaxis and, in general, “dance language” communication among members of the hive occur inside the hive, making these phenomena difficult to observe and study. Therefore, although many impressive results have been obtained concerning sophisticated mechanisms of the “dance language” (see, for example: [17,28]), the role of antennae in the communication of honeybees remains so far obscure.

Evidence of lateral biases in another huge group of eusocial insects – ants (Insecta, Hymenoptera, Formicidae) – has been reported at the level of collective orientation [12]. Twelve ant species *Lasius* spp. appeared to keep mainly to the right side of their foraging “streets”, whereas there was only one species that kept to the left. On streets in trees, 49 *Lasius niger* colonies kept to the right versus 26 to the left. Besides, in this ant species a significant majority of couples in the laboratory had the left side of their bodies exposed to their partners when resting. This identical left body side exposure when resting and foraging in streets also correctly predicted that solely foraging *L. niger* would turn to the right significantly more often than to the left (the ratio was 14:2) since the right side of the brain has been shown to be involved in foraging in many species [32].

Ants have been largely studied especially as model for investigating natural communication of animals based on the ideas of

information theory [21,23]. Long term investigations based on the “binary tree” experimental paradigm have demonstrated highly social red wood ant species as being able to pass exact information to their nest mates by means of distant homing, i.e. messages about remote events come from the scouting individual, without other cues such as scent trail or direct guiding. The binary tree experimental paradigm is designed to study the process of information transfer in ants. The basic idea is that ants are forced to communicate the information about the location of a food source within the maze; the quantity of this information is easy to measure and it can be controlled by experimenters [18]. However, although “antennal code” (sensu: [35]) was considered among the main means of distant homing in red wood ants [20] the “binary tree” experiments had nothing to do with antennal contacts themselves, being aimed at investigation of characteristics of “ant language” without any attempts to decipher signals [27]. What is of particular importance for our study is that applying of the “binary tree” paradigm allows to observe strong division of labour in red wood ants between scouting individuals and members of their constant teams (“foragers”), and to distinguish between different kinds of antennal contacts [20,21]. Summarizing “binary tree” studies, at least three types of antennal contacts can be distinguished: (1) “feeding” contacts where a “donor” (D) ant is exchanging food with a “receiver” (R) ant through trophallaxis; (2) “simple” information contacts that look (and work) very much like the “round dance” in honeybees: their goal is to activate foragers; and (3) “true” information contacts that serve for transferring concrete information, like the “waggle dance” in bees and precede the purposeful trip of the “compact” foraging group in ants. It is worth noting here that, unlike in honeybees, each scouting ant attracts to the feeder only members of its constant team (see details in [19]).

In this study we focused on the “feeding” contacts, as the first step to examine possible asymmetry in antennal use during ant communication. We observed that the “receiver” ant used the right antenna significantly more often than the left one during the “feeding” contacts with the “donor” ant. As far as we know, this is the first evidence of lateralization in antennal contacts in ants.

2. Methods

2.1. Subjects

A colony of red wood ants *Formica aquilonia* Yarrow consisting of about 2000 individuals was used in these experiments. The ants were housed in plexiglass nests (10 cm × 15 cm × 12 cm) and fed once in two or three days, only in the maze. All actively foraging ants were individually marked with coloured paint (see [20], for details).

2.2. Experimental procedures

We used the “binary tree” experimental paradigm suggested by Reznikova and Ryabko [23]. Briefly, in the maze “binary tree” each “leaf” of the “tree” ended with an empty trough, except one with syrup. In this situation a scouting ant should transmit to its nestmates the information about a sequence of turns. It is technically significant for our study that the use of the “binary tree” paradigm makes contacts between a scouting ant and constant members of its team (foragers) observable on the laboratory arena, outside the ant nest because, as it was revealed earlier, after 2–3 sole trips of a scout towards a feeder foragers usually leave the nest and wait for their scout outside [21,23].

In our experiment the number of forks of the binary tree was 3 (Fig. 1). To prevent the access to food in a straight line, the labyrinth was placed in a bath (600 mm × 600 mm) with water. To avoid the use of an odour track the experimental set-up was replaced by an identical one when the scout was in the nest or on the arena contacting its group (Fig. 1; see details in [20]).

Reproducing the “binary tree” experiments in our study, we found out that both “true information” and “simple information” types of contacts between scouting ants (also playing the role of donors, D) and foragers (receiver ants, R) were difficult to observe and analyse because, as this was described earlier (see: [20]), a scout performed contacts with several members of its team simultaneously, and video camera fixed fast movements of up to four pairs of antennae (Fig. 2).

As a first step of studying lateralization of antennal contacts during communication in ants, we focused mainly on “feeding” contacts through trophallaxis and those

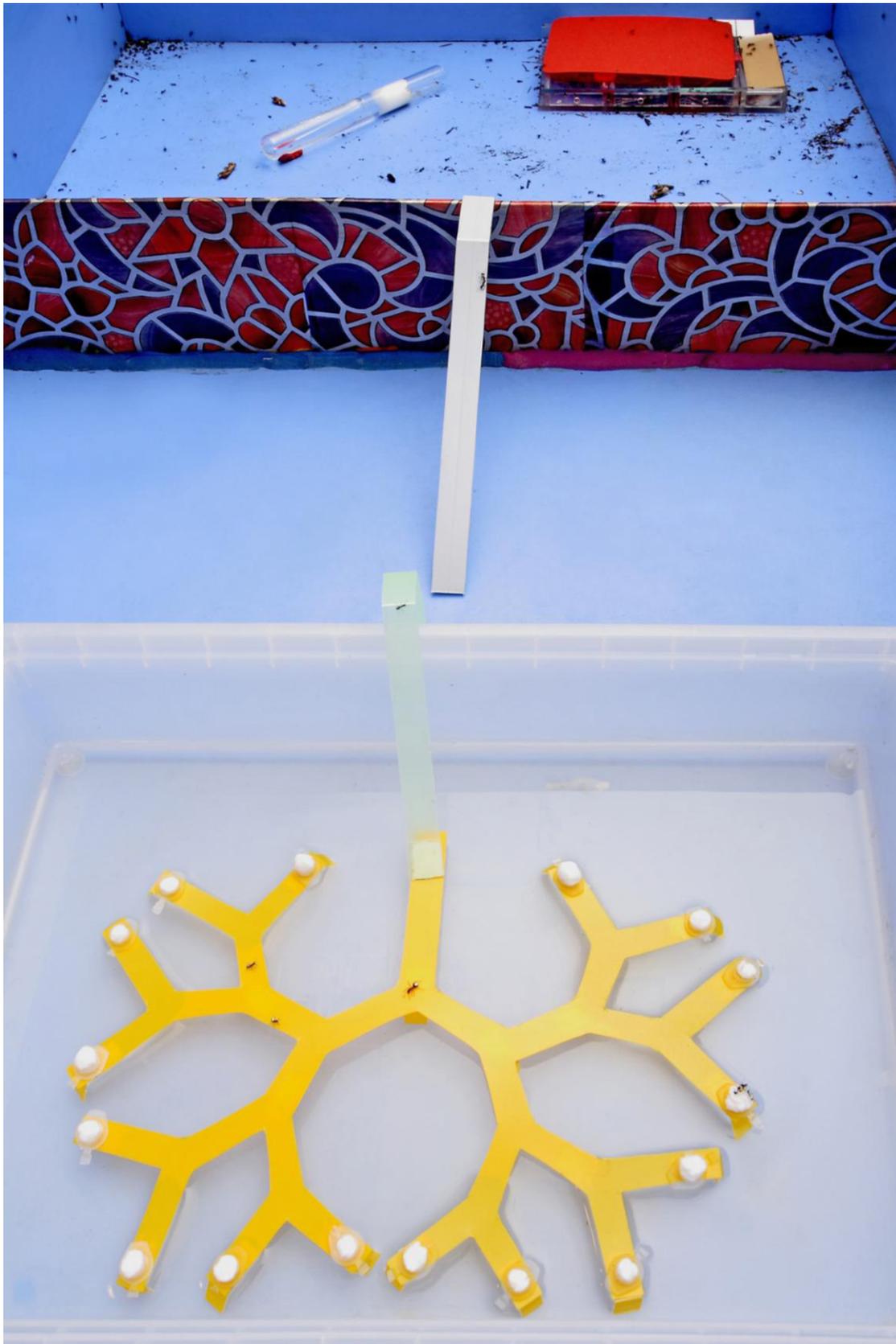


Fig. 1. A laboratory arena divided into two parts, one containing a laboratory nest, and another with a binary tree with 3 forks. Photograph by N. Bikbaev.



Fig. 2. Three “receiver” (R) ants contacting simultaneously with their “donor” (D) ant.

Photograph by N. Bikbaev.

“simple” information contacts in which D–R pairs contacted separately (Fig. 3; also see Supplementary Materials).

Supplementary material related to this article found, in the online version, at doi:10.1016/j.bbr.2012.03.014.

We video-recorded antennal contacts between the D ant and the R ant, when the D ant was coming back from the maze, using a Sony HandyCam DCR-SX44E. We recorded 141 encounters between ants returning from the binary tree and those that were waiting them outside the nest. From them we selected 58 video records of good quality (not wobble). We rejected video records of group contacts of ants and we chose 27 records in which pairs of ants are interacted. Among these 27 records we selected 10 video records in which pair contacts of ants were clearly seen and long enough to count touches of right and left antennae. So we selected 8 encounters between ants performing “feeding” contacts (266 s in total) and 2 additional videos of “simple” information contacts (35 s).

2.3. Statistical analyses

All video recording was analysed using VLC Media player 2.0.0 (VideoLAN Project, France) at 0.12× speed by two experimenters. The number of contacts made by the right and the left antenna of the R ant on the D ant’s head and the number of contacts made by the right and the left antenna of the D ant on the R ant’s head were counted (see Fig. 3). Although the number of touches recorded by the two experimenters was the same in most of the case, we considered the mean value between the number of antennal contacts recorded by one experimenter and the number of antennal contacts recorded by the second experimenter. The inter-rater reliability between the two recorders was evaluated with a two-tailed Spearman’s rank correlation coefficient (Spearman’s $\rho = 0.964$; $P < 0.0001$).

For both R and D ants and for both antennae, the mean number of touches was normalized on the number of total touches. Lateral asymmetries in the antennal use were computed using the index: $LI = (R - L / R + L)$, where R and L indicate, respectively, the normalized mean number of times in which each ant use the right or the left antenna during the antennal contacts. Hence a score of 1.0 indicated exclusive use of the right antenna and a score of -1.0 indicated exclusive use of the left antenna. An LI score of 0 indicated equal numbers of right and left antenna touches. Significant departures from chance level (0) were estimated by two-tailed one-sample t tests.

For all statistical tests, SPSS software (SPSS Inc., Chicago, IL, USA) was used, and the results were considered statistically significant for $P < 0.05$.

3. Results

For “feeding” contacts, data are plotted in Fig. 4, where the normalized mean number of times in which R ant (Fig. 4a) and D ant (Fig. 4b) used the right or the left antenna during the antennal contacts is plotted.

Laterality index values for R ant’s antenna use are shown in Fig. 5a. “Receiver” ants consistently used their right antenna more often than the left antenna during the antennal contacts with the “donor” ants (two-tailed one-sample t test: $t_7 = 3.768$, $P = 0.007$). Laterality index values for D ant’s antenna use are shown in Fig. 5b. “Donor” ants did not show any antenna preference during



Fig. 3. Pair feeding contacts between the “receiver” (R) ant and the “donor” (D) ant. The D is left on top.

Photographs by N. Bikbaev.

the antennal contacts with R ants (two-tailed one-sample t test: $t_7 = -0.366$, $P = 0.725$, n.s.).

For “simple” information contacts, the sample is too small for a proper statistical analysis. However, we find interesting to underline that both “receiver” and “donor” did not show any antennal preference. (Two-tailed one-sample t test for R ants: $t_1 = 2.333$, $P = 0.258$, n.s.; for D ants: $t_1 = -0.435$, $P = 0.739$, n.s.)

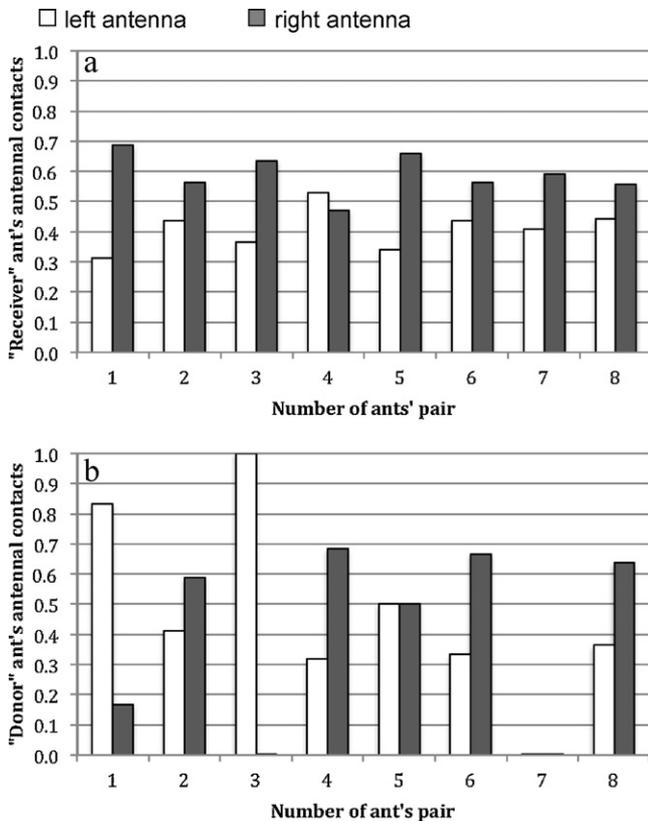


Fig. 4. Normalized mean number of antennal touches made by “receiver” (R) ant (a) and by “donor” (D) ant (b) with the left antenna (white bars) and with the right antenna (dark grey bar) during “feeding” contacts for the 8 ant's pairs analysed.

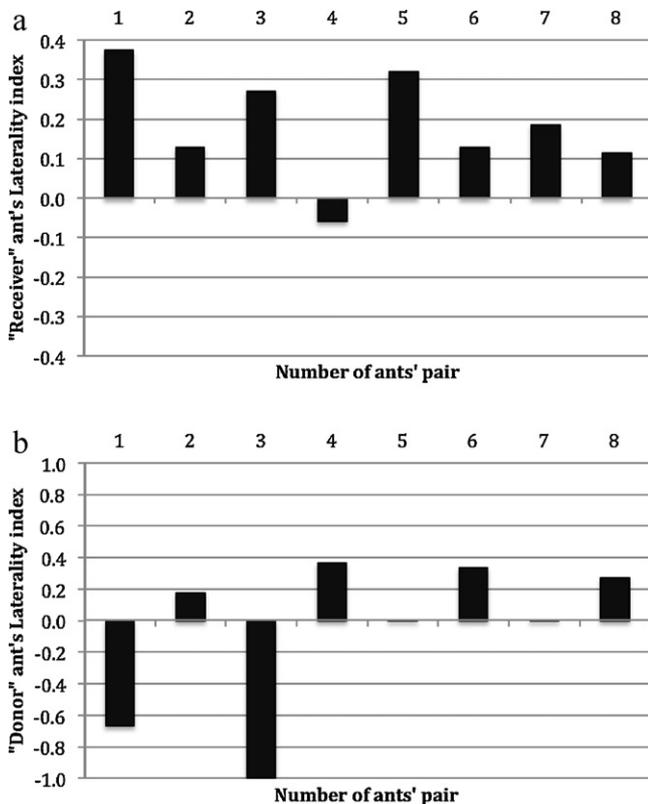


Fig. 5. Laterality index values for antennal use of “receiver” (R) ant (a) and “donor” (D) ant (b) during “feeding” contacts for the 8 ant's pairs analysed.

4. Discussion

In the “feeding” contacts “receiver” ants showed a significant lateral bias towards the right antenna during their touches with “donor” ants. The same asymmetry was not apparent in the number of touches made by “donor” ants. In “simple” information contacts, neither receiver ants nor donor ants revealed any lateralization in the antennal contacts. Even if it will be very interesting to analyse the asymmetry observed at a deeper level, the main result at this stage is the finding of lateralization in antennal contacts of the ants themselves, which is novel, as far as we know.

The finding of lateralization in the antennal contacts during trophallaxis is very interesting also because it is the first evidence of a population-level asymmetry in a natural social behaviour in eusocial insects, although it remains to be investigated how the observed asymmetry works in terms of effectiveness of communication. Male field crickets, *Gryllus campestris* (Insecta, Orthoptera, Gryllidae), for example, show physiological asymmetry in wing orientation during singing: they use the right forewing more commonly than the left [4]. If this wing orientation is inverted (i.e. the left wing lies over the right), during stridulation, almost no sound is produced [the sound intensity is 100 times less – 4] although the wings are morphologically identical [29]. Interestingly, a special wing-spreading behaviour is used by the insect to restore the normal right over left orientation and thus to ensure an effective acoustical communication. A set of hair plates in the sub-costal region of the wings seems to play an important role in preventing the inversion of the wings during stridulation, which ensures the high intensity sound production necessary to attract females [4]. A similar hypothesis may be addressed for the asymmetry observed here in “feeding” antennal contacts in ants: to have an effective trophallaxis the “receiver” ant should interact with the donor using its right antenna more than its left antenna. The reason behind this idea may be that, as in honeybees [6], the right antenna presents more olfactory/taste receptors and so it may play a role during trophallaxis. It would be also interesting to analyse lateralization of antennae in ants when they “ask” aphids for drops of honeydew. Kloft [13] compared the aphid's abdomen to the head of an ant offering liquid food, and Reznikova and Novgorodova [22] have shown that naïve red wood ants used the same stereotypic repertoires of movements during trophallaxis and aphid milking. It is very likely that the innate universal behavioural stereotype lies in the basis of begging for food in ants during inter- and intraspecific communication.

Overall, the asymmetry observed in the antennal use by the “receiver” ant during “feeding” contacts with a “donor” ant seems to support the hypothesis predicted by mathematical models on the evolution of lateralization suggesting that the alignment of lateralization at the population-level matters in social interactions [9,10].

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References

- [1] The nature of behavioral lateralization in the chick. Andrew RJ, editor. Neural and behavioral plasticity. The use of the chick as a model. UK: Oxford University Press; 1991. p. 536–54.
- [2] Anfora G, Frasnelli E, Maccagnani B, Rogers LJ, Vallortigara G. Behavioural and electrophysiological lateralization in a social (*Apis mellifera*) but not in a non-social (*Osmia cornuta*) species of bee. Behav Brain Res 2010;206(2):236–9. doi:10.1016/j.bbr.2009.09.023.

- [3] Bitterman ME, Menzel R, Fietz A, Schafer S. Classical conditioning of proboscis extension in honeybees (*Apis mellifera*). *J Comp Psychol* 1983;97:107–19, doi:10.1037/0735-7036.97.2.107.
- [4] Elliott CJH, Koch UT. Sensory feedback stabilizing reliable stridulation in the field cricket *Gryllus campestris* L. *Anim Behav* 1983;31:887–901, doi:10.1016/S0003-3472(83)80244-9.
- [5] Frasnelli E, Vallortigara G, Rogers LJ. Response competition associated with right–left antennal asymmetries of new and old olfactory memory traces in honeybees. *Behav Brain Res* 2010;209:36–41, doi:10.1016/j.bbr.2010.01.014.
- [6] Frasnelli E, Anfora G, Trona F, Tassarolo F, Vallortigara G. Morpho-functional asymmetry of the olfactory receptors of the honeybee (*Apis mellifera*). *Behav Brain Res* 2010;209:221–5, doi:10.1016/j.bbr.2010.01.046.
- [7] Frasnelli E, Vallortigara G, Rogers LJ. Right–left antennal asymmetry of odour memory recall in three species of Australian stingless bees. *Behav Brain Res* 2011;224(1):121–7, doi:10.1016/j.bbr.2011.05.026.
- [8] Frasnelli E, Vallortigara G, Rogers LJ. Left–right asymmetries in behaviour and nervous system among invertebrates. *Neurosci Biobehav Rev* 2012;36:1273–91, doi:10.1016/j.neubiorev.2012.02.006.
- [9] Ghirlanda S, Vallortigara G. The evolution of brain lateralization: a game theoretical analysis of population structure. *Proc R Soc Lond B Biol Sci* 2004;271:853–7, doi:10.1098/rspb.2003.2669.
- [10] Ghirlanda S, Frasnelli E, Vallortigara G. Intraspecific competition and coordination in the evolution of lateralization. *Philos Trans R Soc Lond B Biol Sci* 2009;364:861–6, doi:10.1098/rstb.2008.0227.
- [11] Güntürkün O, Diekamp B, Manns M, Nottelmann F, Prior H, Schwarz A, et al. Asymmetry pays: visual lateralization improves discrimination success in pigeons. *Curr Biol* 2000;10:1079–81, doi:10.1016/S0960-9822(00)00671-0.
- [12] Heuts BA, Cornelissen P, Lambrechts DYM. Different attack modes of *Formica* species in interspecific one-on-one combats with other ants (Hymenoptera: Formicidae). *Ann Zool (Wars)* 2003;53:205–16.
- [13] Kloft W. Die Trophobiose zwischen Waldameisen und Pflanzenläusen mit Untersuchungen über Wechselwirkungen zwischen Pflanzenläusen und Pflanzengewebe. *Entomophaga* 1960;5:43–54.
- [14] Letzkus P, Ribi WA, Wood JT, Zhu H, Zhang SW, Srinivasan MV. Lateralization of olfaction in the honeybee *Apis mellifera*. *Curr Biol* 2006;16:1471–6, doi:10.1016/j.cub.2006.05.060.
- [15] Levy J. The mammalian brain and the adaptive advantage of cerebral asymmetry. *Ann NY Acad Sci* 1977;299:264–72, doi:10.1111/j.1749-6632.1977.tb41913.x.
- [16] McGrew WC, Marchant LF. Laterality of hand use pays off in foraging success for wild chimpanzees. *Primates* 1999;40:509–13, doi:10.1007/BF02557586.
- [17] Michelsen A, Anderson BB, Kirchner WH, Lindauer M. Honeybees can be recruited by a mechanical model of a dancing bee. *Naturwissenschaften* 1989;76:277–80, doi:10.1007/BF00368642.
- [18] Reznikova Zh. Animal intelligence: from individual to social cognition. UK: Cambridge University Press; 2007.
- [19] Reznikova Zh. Dialog with black box: using Information Theory to study animal language behaviour. *Acta Ethol* 2007;10:1–12.
- [20] Reznikova Zh. Experimental paradigms for studying cognition and communication in ants (Hymenoptera: Formicidae). *Myrmecol News* 2008;11:201–14.
- [21] Reznikova Zh. Division of labour and communication at the individual level in highly social *Formica* ants (Hymenoptera, Formicidae). *Russ Entomol J* 2011;20(3):315–9.
- [22] Reznikova ZhI, Novgorodova TA. The importance of individual and social experience for interaction between ants and symbiotic aphids. *Dokl Biol Sci* 1998;359:173–5.
- [23] Reznikova Zh, Ryabko B. Experimental study of the ants communication system with the application of the Information Theory approach. *Memorabilia Zool* 1994;48:219–36.
- [24] Rogers LJ. Advantages and disadvantages of lateralization. In: Rogers LJ, Andrew RJ, editors. Comparative vertebrate lateralization. UK: Cambridge University Press; 2002. p. 126–53.
- [25] Rogers LJ, Vallortigara G. From antenna to antenna: lateral shift of olfactory memory in honeybees. *PLoS ONE* 2008;3:e2340, doi:10.1371/journal.pone.0002340.
- [26] Rogers LJ, Zucca P, Vallortigara G. Advantage of having a lateralized brain. *Proc R Soc Lond B Biol Sci* 2004;271:S420–2, doi:10.1098/rsbl.2004.0200.
- [27] Ryabko BYa, Reznikova ZhI. Using Shannon Entropy and Kolmogorov Complexity to study the communicative system and cognitive capacities in ants. *Complexity* 1996;2:37–42.
- [28] Seeley TD. The wisdom of the hive. US: Harvard University Press, Cambridge, MA; 1995, p.302.
- [29] Stärk AA. Untersuchungen am Lautorgan einiger Grillen- und Laubheuschrecken-Arten, zugleich ein Beitrag zum Rechts-Links-Problem. *Zool Jahrb Anat* 1958;77:9–50.
- [30] Vallortigara G. Comparative neuropsychology of the dual brain: a stroll through left and right animals' perceptual worlds. *Brain Lang* 2000;73:189–219, doi:10.1006/brln.2000.2303.
- [31] Vallortigara G. The evolutionary psychology of left and right: costs and benefits of lateralization. *Dev Psychobiol* 2006;48:418–27, doi:10.1002/dev.20166.
- [32] Vallortigara G, Rogers LJ. Survival with an asymmetrical brain: advantages and disadvantages of cerebral lateralization. *Behav Brain Sci* 2005;28:575–633, doi:10.1017/S0140525X05000105.
- [33] Vallortigara G, Chiandetti C, Sovrano VA. Brain asymmetry (animal). *WIREs Cognit Sci* 2011;2:146–57, doi:10.1002/wcs.100.
- [34] von Frisch K. The dance language and orientation of bees. Cambridge, MA: US: Harvard University Press; 1967.
- [35] Wasmann E. Die psychischen Fähigkeiten der Ameisen. *Zoologica* 1899;26:1–133.