

Research



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Author for correspondence:

Elizabeth A. Tibbetts
e-mail: tibbetts@umich.edu

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Animal behaviour

Transitive inference in *Polistes* paper wasps

Elizabeth A. Tibbetts, Jorge Agudelo, Sohini Pandit and Jessica Riojas

Ecology and Evolution, University of Michigan, Ann Arbor, MI 48109, USA

EAT, 0000-0002-5625-892X

Transitive inference (TI) is a form of logical reasoning that involves using known relationships to infer unknown relationships ($A > B$; $B > C$; then $A > C$). TI has been found in a wide range of vertebrates but not in insects. Here, we test whether *Polistes dominula* and *Polistes metricus* paper wasps can solve a TI problem. Wasps were trained to discriminate between five elements in series (A_0B^- , B_0C^- , C_0D^- , D_0E^-), then tested on novel, untrained pairs (B versus D). Consistent with TI, wasps chose B more frequently than D. Wasps organized the trained stimuli into an implicit hierarchy and used TI to choose between untrained pairs. Species that form social hierarchies like *Polistes* may be predisposed to spontaneously organize information along a common underlying dimension. This work contributes to a growing body of evidence that the miniature nervous system of insects does not limit sophisticated behaviours.

1. Introduction

During transitive inference (TI), animals use known relationships to deduce unknown relationships [1]. Theoretical and empirical work suggests that species with complex social behaviour will be more adept at TI than species with less complex social behaviour [2,3]. TI may be favoured in social species because it allows animals to keep track of social relationships. Consistent with this hypothesis, social complexity is linked with the capacity for TI [4,5]. For example, highly social pinyon jays have more accurate TI abilities than less social Western scrub-jays [4].

The capacity for TI has been tested in one invertebrate, the honeybee *Apis mellifera* [6]. Benard & Giurfa found honeybees do not make TI, perhaps because their small nervous system imposes cognitive constraints that limit the capacity for this type of reasoning. Honeybee brains have approximately one million neurons [7]. This is tiny compared with 85 billion neurons in the human brain [8] or 1 billion neurons in some birds [9]. Alternatively, honeybees may not perform TI because this ability is not useful in honeybee societies. Although honeybees live in large social groups, worker bees do not reproduce or form dominance hierarchies. Instead, workers use group-level recognition and self-organization to produce apparently complex behaviour with little individual cognitive investment [10,11].

Here, we test whether *Polistes dominula* and *Polistes metricus* wasps can solve a TI problem. *Polistes* provide a good model to test whether an insect's small nervous system limits the capacity for TI, as *Polistes* and *Apis mellifera* brains are similarly sized [12]. However, *Polistes* have the type of complex social behaviour thought to involve TI in vertebrates [13,14]. *Polistes* often live in cooperative societies where nest-founding queens compete to form linear dominance hierarchies. In the early spring, *Polistes* nest-founding queens compete with many rivals on and off nests before settling down in stable, hierarchical groups. A wasp's rank in the hierarchy determines shares of reproduction, work and food [15].

We tested TI using the most widely used method: a five-element training procedure [1,16]. Wasps were trained with five colours (arbitrarily labelled A, B, C, D, E). First, wasps were trained to discriminate four pairs of colours (A₀B-, B₀C-, C₀D- and D₀E-; -, electric shock, 0 no shock). Then, individuals were tested on novel pairs without training (B versus D, A versus E). If wasps organize the trained stimuli into the implicit hierarchy A > B > C > D > E and use TI to choose between untrained pairs, they will choose B more frequently than D and A more frequently than E.

2. Material and methods

Foundresses used in this study were collected near Ann Arbor, MI. Foundresses and their nests were housed in the laboratory with ad lib sugar and *Galleria mellonella* caterpillars.

TI was tested by means of the widely used five-element training procedure [1,16]. Wasps were trained on four pairs of colours in series: A₀B-, B₀C-, C₀D- and D₀E-, using a procedure modified from [17]. The colours used during training (electronic supplementary material, figure S1) were chosen because they are easy for wasps to discriminate [18]. Wasps learned all colours similarly and colours were assigned a unique order for each wasp to ensure specific colours could not influence experimental outcomes. Stimuli were printed on a Sony Color Station Printer. Wasps were trained and tested on one pair a day for 4 days. The order of trained pairs was varied. Half the wasps were trained to differentiate A versus B on day 1 and D versus E on day 4 (7 *P. metricus*, 12 *P. dominula*). Half the wasps were trained to differentiate D versus E on day 1 and A versus B on day 4 (10 *P. metricus*, 11 *P. dominula*).

During training, the wasp was placed in the middle of a 3 × 10 × 0.7 cm rectangle (electronic supplementary material, figure S2). One end of the rectangle had the correct stimulus and the other end of the rectangle had the incorrect stimulus. The entire floor of the rectangle was electrified at 0.4 V except the 2.25 cm closest to the correct stimulus, the 'safety zone'. At the start of training, the rectangle had two clear plastic partitions confining the wasp to the centre. The wasp was placed in the centre, the electric shock was turned on, both partitions were removed simultaneously, and the wasp was free to walk throughout the arena for 30 s. Wasps entered the safe zone in every trial. After 30 s, wasps were removed and given a 2 min break in a holding container. The placement of the negative and neutral stimuli (right or left side) was determined randomly and changed between trials. Six 30 s training trials were run. Then, the wasp was given a 45 min break in a holding container before we tested learning accuracy.

During testing for the premise pairs, we recorded whether the focal wasp chose the correct or incorrect stimulus over 10 trials. Wasps were removed from the arena as soon as they made a choice, typically 5 s. The wasp made a choice when its head and thorax moved beyond the partitions placed 2.5 cm from the end of the rectangle. After removal, the wasp was given a 1 min break in the holding container. Testing alone is not sufficient for learning. When wasps were tested on pairs of colours without the initial training, they performed no better than chance (*P. dominula* $n = 14$, mean = 5.1, $p = 0.92$; *P. metricus* $n = 14$, mean = 4.8, $p = 0.67$).

On day 5, training was refreshed with one 30 s training trial for each premise pair. Then, wasps were given a 45 min break before being tested on the untrained inferential pairs (B versus D and A versus E). The methods for testing premise pairs and inferential pairs were similar, with two key differences. First, the floor of the arena was electrified during the premise pair test but not during the inferential pair tests. As a result, neither stimulus was reinforced and any preferences are not due to simple associative learning. Second, the trainer was blind to the

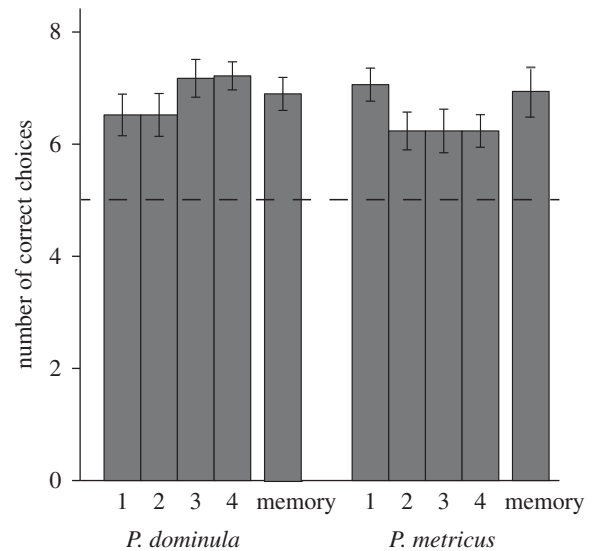


Figure 1. Mean \pm s.e. number of correct choices for the trained premise pairs (1–4) and memory for the first trained pair when retested on day 5. Numbers 1–4 are the order that premise pairs were trained. 'Memory' is accuracy on the first premise pair when retested on day 5 without additional training. The dashed line represents the 50 : 50 random expectation. Wasps performed significantly better than expected by chance on each trained pair (all $p < 0.01$). There was no difference in accuracy across trained pairs or between the initial test and memory.

wasp's training history to ensure they could not unintentionally bias choice toward a particular stimulus. At the end of day 5, wasps were retested on the first premise pair trained (either A₀B- or D₀E-). Retesting was done to assess whether wasps remembered the initial premise pair for the 5-day experiment.

Analyses were performed in SPSS v. 24. Seventeen *P. metricus* and 23 *P. dominula* completed all trials. Whether or not a wasp learned a particular stimulus was tested by comparing the proportion of correct choices with the neutral 0.5 expectation using a one-sample *t*-test. Accuracy across different stimuli was compared using a linear mixed model. Wasp ID was included as a subject variable to control for any similarity in performance in the same wasp. The dependent variable was the number of correct choices (out of 10). In one analysis, the independent variable was the order premise pairs were trained (continuous, 1–4). In the other analysis, the independent variable was the particular stimulus (categorical). *Post hoc* pairwise analyses were not performed because the overall model found no differences in accuracy across stimuli. Memory for the first trained stimulus was tested by comparing accuracy during the initial test on day 1 with the second test on day 5 using a paired *t*-test.

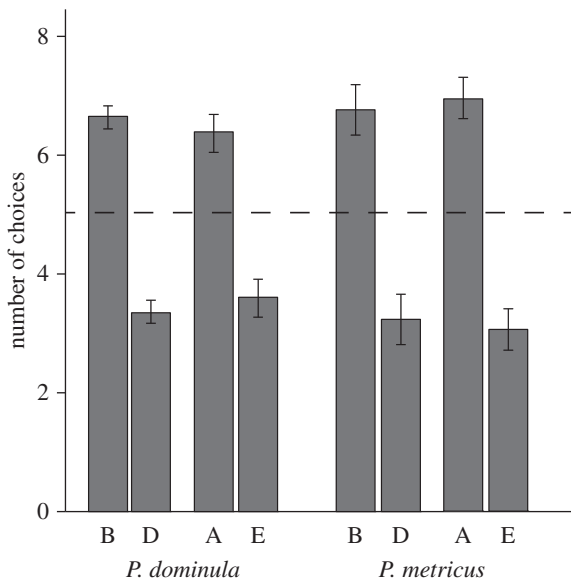
3. Results

Wasps learned all premise pairs, as they chose the correct colour more often than expected by random chance (figure 1 and table 1, all $p \leq 0.01$). Wasps learned premise pairs with similar accuracy, though there was a non-significant trend toward increasing accuracy over time in *P. dominula* (figure 1, $F_{1,90} = 3.3$, $p = 0.07$) and decreasing accuracy after the first pair in *P. metricus* ($F_{1,65} = 2.7$, $p = 0.11$).

After wasps were trained and tested on all four premise pairs, they were tested on novel pairs B versus D and A versus E. Consistent with TI, wasps chose B and A more often than expected by chance (figure 2 and table 1, all $p < 0.01$). Choice accuracy was similar on trained and inferential pairs (*P. dominula* $F_{5,132} = 1.3$, $p = 0.27$; *P. metricus* $F_{3,96} = 1.2$,

Table 1. Results of one-sample *t*-test comparing choices with the neutral 50 : 50 expectation.

	first	second	third	fourth	BD	AE	retest first
<i>P. dominula</i>	$t_{22} = 4.1, p < 0.001$	$t_{22} = 3.9, p = 0.001$	$t_{22} = 6.4, p < 0.001$	$t_{22} = 8.8, p < 0.001$	$t_{22} = 8.5, p < 0.001$	$t_{22} = 4.4, p < 0.001$	$t_{22} = 6.5, p < 0.001$
<i>P. metricus</i>	$t_{16} = 7.4, p < 0.001$	$t_{16} = 3.6, p = 0.002$	$t_{16} = 3.2, p = 0.006$	$t_{16} = 4.2, p = 0.001$	$t_{16} = 4.12, p = 0.001$	$t_{16} = 5.6, p < 0.001$	$t_{16} = 4.5, p < 0.001$

**Figure 2.** Mean \pm s.e. number of choices during tests on untrained, inferential pairs. Dashed line represents the 50 : 50 random expectation. In both species, wasps chose A over E and B over D (all $p \leq 0.001$). These results suggest that wasps organize the trained stimuli into the implicit hierarchy $A > B > C > D > E$ and use TI to choose between untrained stimulus pairs.

$p = 0.30$). The preference for B over D and A over E was not influenced by the order wasps learned the premise pairs. Some wasps were first trained to discriminate A versus B, while others were first trained to discriminate D versus E. Both groups made choices consistent with TI, as they chose B over D and A over E (electronic supplementary material, table S1, all $p < 0.05$).

TI requires individuals to remember the premise pairs for the entire experiment. Wasps do remember the first pair until the end of the experiment, as they performed better than chance when retested on the first premise pair on day 5 (table 1, $p < 0.001$). Further, there was no difference in choice accuracy between the initial test immediately after training on day 1 and the retest on day 5 (*P. dominula*, $t_{22} = 0.7, p = 0.48$; *P. metricus*, $t_{16} = 0.25, p = 0.81$).

4. Discussion

Our results illustrate that paper wasps can recall multiple dyadic relationships and transitively infer relationships among novel pairs of stimuli. Consistent with TI, wasps choose B over D and A over E (figure 2 and table 1). Preference for A over E could be due to direct reinforcement because A was always associated with safety and E was always associated with shock. By contrast, B and D were associated with shock in half the trials and safety in half the trials. As a result, the preference for B over D provides critical evidence that *Polistes* organize the trained stimuli into the implicit hierarchy $A > B > C > D > E$ and use TI

to choose among novel pairs. To our knowledge, this is the first study to show that an invertebrate uses TI.

One unexpected aspect of the results is that *P. dominula* and *P. metricus* performed similarly. We originally predicted *P. dominula* would be more adept at TI than *P. metricus* because *P. dominula* foundresses often nest in large cooperative groups (up to 10 foundresses), while *P. metricus* foundresses nest alone or in small cooperative groups (two foundresses). Perhaps they perform similarly because recent ancestors of *P. metricus* lived in large cooperative groups [19] and the associated cognitive skills have not been lost. Alternatively, TI could be used in multiple social contexts, including competition with multiple potential nest-mates prior to nest foundation [15]. Additional research across species and social contexts will be important to understand how TI evolves.

The one previous study on TI in an invertebrate (honeybee) found very different results [6]. First, honeybees were unable to learn all four trained premise pairs ($A + B_0, B + C_0, C + D_0, D + E_0$), suggesting there may be interference or memory constraints that limit the ability of bees to learn multiple, overlapping discrimination tasks. By contrast, there was no evidence of memory constraints in wasps, as wasps learned all trained pairs equally well (figure 1). Bees also did not build and manipulate the hierarchy $A > B > C > D > E$. Instead, bees made choices based on which stimuli were rewarded most recently as well as the associative strength of the stimuli [6]. By contrast, wasps spontaneously organized the trained pair colours into an interconnected series and made inferences based on that series.

Why do wasps and bees perform so differently? One possibility is that different types of cognitive abilities are favoured in bees and wasps because they have different social behaviour. A honeybee colony has one queen and multiple equally ranked workers. Worker bees rear the queen's offspring and do not aggressively compete over reproduction or rank [20]. By contrast, *Polistes* foundresses live in more socially complex societies where wasps compete with rivals on and off nests, form stable linear dominance hierarchies and have unique roles in their colony [15]. Species like *Polistes* wasps that have complex social lives may benefit by organizing information linearly because this allows individuals to rapidly make deductions about novel social relationships. As a result, socially flexible taxa may be predisposed to spontaneously organize information along a common underlying dimension, as required for TI [3,14]. Future work will be important to test the role of TI during social interactions in *Polistes*.

The performance of wasps is in line with previous studies in vertebrates. In vertebrates, the number of trials required to learn the premise pairs varied across taxa and training methods, from less than 50 to more than 100 [4,16,21]. Wasps learned the premise pairs rapidly, requiring only six 30 s trials. Further, wasps remembered this initial training over the entire 5-day experiment. The performance of wasps and vertebrates is not directly comparable, as wasps

were trained with negative reinforcement while vertebrate studies typically use food rewards. Nevertheless, wasps are surprisingly adept at learning and remembering premise pairs and making inferences.

Originally, TI was regarded as a hallmark of human cognition [1] and was thought to be based on logical deduction. More recently, there have been questions about whether TI requires higher-order reasoning or can be solved with associative processes like value transfer [22,23]. Subsequent work has shown that simple associative processes are not sufficient to account for TI performance [1]. As a result, animals that identify transitive relationships when trained with a five-element training procedure are commonly accepted to be capable of TI [24]. This study illustrates that paper wasps naturally build and manipulate an implicit hierarchy, but it does not test the precise mechanisms that underlie this ability. Future experiments will be useful to assess the cognitive strategies used for TI across taxa.

Overall, the results of this study add to a growing body of evidence that the miniature nervous systems of insects do not

limit sophisticated behaviours [7,25–27]. The capacity for complex behaviour may be shaped by the social environment in which behaviours are beneficial rather than being limited by brain size.

Ethics. All experiments complied with the laws of the United States and international ethical standards.

Data accessibility. The data are included as electronic supplementary material.

Authors' contributions. E.A.T. conceived the study, analysed the data and wrote the manuscript. J.A., S.P. and J.R. performed the research and participated in manuscript preparation. All authors gave approval of the final version and agree to be accountable for all aspects of the work.

Competing interests. The authors declare no competing interests.

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