

# Sensitivity to Number Parity in Young *Myrmica sabuleti* Ants

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## Abstract

Young *Myrmica sabuleti* worker ants aged at most a few weeks and settled in small single-cohort colonies, were learned, by operant conditioning, to recognize either 2 or 3 dots displayed on a stand. When tested in front of two stands bearing different numbers of dots, one even and the other odd, their response was always higher towards the stand bearing the same parity as the one learned. They thus reacted to the parity corresponding to the number of dots they have learned to associate with the reward. These young ants were sensitive to the parity of a number of dots ranging from 1 to at least 6, but could not discriminate their parity when in front of 5 and 6. They were somewhat more responsive towards an even number than towards an odd number, having learned a number with this parity. A preceding study showed that older workers (experienced foragers) are sensitive to the parity of numbers of dots ranging from 1 to at least 7, and this equally whether the numbers are odd or even. Their limit of parity discrimination of close numbers is in front of 6 and 7. It therefore appears that, although present early on, the sensitivity of young workers to parity of numbers of elements and to number discrimination has to improve over the course of their lives, perhaps through an age-related maturation process, or through acquired experience.

**Keywords:** behavioral maturation, innate behavior, number parity recognition, operant conditioning

## 1. Introduction

The workers of the ant *Myrmica sabuleti* Meinert, 1861 have been proved to be sensitive to the parity of numbers of items. Trained to an even or an odd number *versus* an odd or an even number, when tested in front of different pairs of even and odd numbers, they responded to the number having the same parity as that to which they were trained (Cammaerts & Cammaerts, 2023). It remained to know if this sensitivity is already detained by the young ants living inside of the nest and having not yet gone outside of it, or will be acquired when they become foragers, through for example an age-linked maturation process or the benefit of experience gained outside of the nest. The workers of *M. sabuleti* have a number line as they locate the numbers on a left to right oriented line (Cammaerts & Cammaerts, 2020a); they show this numerosity ability early in their workers' life, as soon as during their first or second month (Cammaerts & Cammaerts, 2020b). *M. sabuleti* workers also have the notion of zero (Cammaerts & Cammaerts, 2019), but this numerosity characteristic is acquired in the course of their experience while foraging, i.e., it is not held by ants only a few weeks old (Cammaerts & Cammaerts, 2020c). We could thus not guess if the sensitivity to the numbers' parity in this ant is innate or acquired during aging. Before relating our experimental work, we recall some information on the parity sensitivity detained by humans and recently demonstrated in bees.

Humans can distinguish odd and even numbers. There is a gradation in the intensity of this ability, being initially, natively, not pronounced. Then, it is understood (at least for the even numbers), and later, it is learned through the numbers' divisibility by two (Berch, Foley, Hill & Ryan, 1999). In number-parity judgments, parity was shown to have no influence until age 12 (Miller & Gelman, 1983), this skill requiring an appropriate pedagogic approach (Zazkis, 1998). Working with odd numbers is slower than working with even numbers (Hines, 1990). Number magnitude is more precociously acquired than complete number parity (Miller & Gelman, 1983). A representation of number magnitude is automatically accessed when judging the parity of Arabic numbers, the magnitude being represented by the numbers' left-right spatial coordinates (Dehaene, Bossini & Giroux, 1993). This representation later on named 'number line' is thus addressed to make a correct parity decision (Reynvoet & Brysbaert, 1999).

Honeybees have been shown to be able to categorize perceived visual elements (Bernard, Stach & Giurfa, 2006). Moreover, trained to 1 to 10 elements of up to 4 different shapes, they could learn to discriminate the even and the odd elements among 2 to 8 of them, and could extrapolate this parity discrimination to 11 and 12 elements (Howard, Greentree, Avargu ̇s-Weber, Garcia, Greentree & Dyer, 2022).

Having previously shown that older, experienced *M. sabuleti* foragers can recognize parity in numbers (Cammaerts & Cammaerts, 2023), we here investigate the innate or acquired nature of this faculty in young workers, as well as their numerical limit of discrimination of nearby numbers. Our experimental protocol is close to that used previously, which inevitably implies some methodological similarities.

## 2. Methods

### 2.1 Collection of Ants; Maintenance of Young Ants

The experiments were performed on the young ants of four colonies of *Myrmica sabuleti* Meinert, 1861 collected in September 2022 from an abandoned quarry located in the Aise valley (Ardenne, Belgium). These four colonies, settled in artificial nests made of a glass tube (see Figure 2 in Cammaerts & Cammaerts, 2023), contained about 500-600 workers, a queen and brood.

By taking young workers who had not yet left the nest, i.e., those who functioned as nurses, immobile on the brood and recognizable by their pale coloring, four new small single-cohort colonies were formed, each one containing only 20 to 30 young workers of their initial colony and perhaps a few larvae they had held between their mandibles. Each of these small colonies was maintained in a small tube half-filled with water, a cotton-plug separating the ants from the water. This nest tube was deposited in a tray (21 cm x 15 cm x 7 cm, Figure 1), the sides of which having been slightly covered with talc. This tray served as foraging area: food was placed inside of it, and the ants leaving the nest tube were trained in it. It is known that the young ants essentially eat sugared solutions, while larvae essentially consumed meat food. The food consisted in a water solution of milk, saccharose (15%) and honey (5%) imbibing a small cotton ball (1 cm<sup>3</sup>) and permanently provided on a small glass piece set at about 8 to 10 cm from the nest entrance. The cotton was refreshed each two days and renewed every seven days. The temperature of the room permanently equaled 20 ° ± 2 °C and the relative humidity about 80%. The luminosity varied between 110 and 330 lux. The electromagnetism had an intensity of 2 μWm<sup>2</sup>. All these conditions were suitable for the used species.

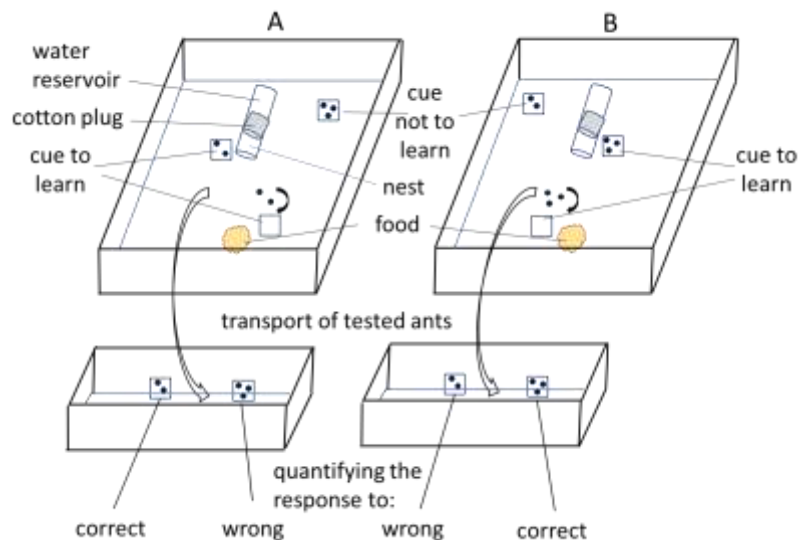


Figure 1. Experimental design used for knowing if the ants' parity sensitivity is innate or acquired

Young ants were maintained in small nests and trained in their foraging area to an even or odd number *versus* an odd or even number. They were tested in a separate small tray in front of even and odd numbers identical (preliminary tests, as shown here for colonies A and B) or partly or fully differing from those presented during training (definitive tests).

### 2.2 Cues Presented to Young Ants

The cues presented to the ants were black circles (dots) drawn on white paper inside a square (2 cm x 2 cm), using Corel Draw® software. Each square was cut and tied on the front face of a vertical stand of the same size, made in strong white paper (Steinbach®) and maintained in position by two folded foots of 1.0 x 0.5 cm. The number of dots

varied according to the experiment performed. To avoid as much as possible confusion variables, the cumulative surface of the dots of each cue was always that of 5 circles of 3.5 mm in diameter and their area perimeter, the same on all cues. The squares bearing the dots were tied to their stand one day before making the experiments for avoiding any remaining odor. The cues with their number of dots, position in the foraging area and daily testing schedule are shown in Figure 2.

### 2.3 Experimental Design

The experimental design is schematized in Figure 1. Three days after having been settled in their new nest, the young workers began to explore their new foraging area in search for food. They were then trained while walking between the nest entrance and the food or elsewhere outside their nest. For this purpose, two identical cues, bearing a number of dots of which the parity has to be learned and memorize were placed, one at the nest entrance, another one near the food, thus at two locations associated with rewards. A cue (only one cue) bearing another number of dots of which the parity has not to be learned was deposited far from any reward (far from the nest entrance). This training was continued along the entire experimental work, during which tests were conducted each day.

Testing was done on two colonies at a time, each colony having its own testing tray (15 cm x 7 cm x 5 cm, which inner side was slightly talked) in which two cues were placed, one being the cue expected to have been learned (the ‘correct’ cue), the other cue with a different parity, expected to have not been learned (the ‘wrong’ cue). The two cues were placed at six cm from each other, the ‘correct’ one on the left for colonies A and C, and on the right for colonies B and D. To collectively test the ants of a colony, ten ants were picked in the foraging area and in the area of the nest entrance and, using a cup, transported to their testing tray. A few seconds after this transport, the ants sighted in front of each presented cue were counted 20 times over 5 minutes, then the ants were transported back at the nest entrance. The cues used to test the ants were identical to or different from those used to train the ants, but were each time novel, never used.

### 2.4 Experimental Planning

This is summarized in Figure 2 and detailed in Table 1.

During three days, the young ants of colonies A or B were trained to 2 or 3 black dots (the ‘correct’ cues to learn and memorize) *versus* 3 or 2 such dots (the ‘wrong’ cues). To check if the correct cues were dully learned, the ants were tested twice on each of these preliminary training days in front of these two kinds of cues. Thereafter, while still being trained to these cues, they were tested each eight hours, on each of the following days 4 to 8, in front of pairs of cues generally differing from the one seen during training by their parity, number and layout of their dots.

The young ants of colonies C or D were trained in the same way, but as experimenting on colonies A and B showed that learning was effective from the first day, the preliminary testing sessions were limited to one day (day 9). After that, while still being trained to these cues, they were tested each eight hours, on each of the following days 10 to 12, in front of pairs of cues generally differing from the one seen during training by the parity, number and layout of their dots.

### 2.5 Statistical Analysis

Using Statistica<sup>®</sup> v10 software, a parametric ANOVA was performed to compare the proportions of correct responses observed during the 18 checking tests carried out during the training period of the first days with those obtained in the definitive tests that were conducted thereafter. The use of a parametric test is justified in the Results section by the conditions of the sample parameters.

Taking into account the pseudo-replications made in the presence of the same pairs of cues with ants of the same colony, a GLMM analysis was performed on a set of 33 testing sessions, i.e., those whose stands did not carry the number of dots the ants had learned or a pair of numbers beyond their discrimination ability. It concerns the tests done on days 5, 6, 8 (this last only with colony B) and 10 to 12. Therefore, the lme4 and MASS packages of v4.1.2 of the R statistical analysis environment (R Core Team, 2021) was used. The dependent variable was the weighted proportion of ants that choose either the even or odd number in response to the parity of the learned number. As for the independent variables, the fixed effects were ‘treatment’ (the learned parity, even or odd) or ‘relation’, the kind of numerical relation between the dots of the two presented cues (i.e., either ‘pair’, the combination of the number of dots of the two cues, or ‘max’, the greatest number of dots of one of the two cues, or ‘ratio’ or ‘mean’ of the two presented numbers or ‘relative difference’, the difference in number of dots between the two cues divided by their mean). The random effect concerned the repetition of measurements made for each test (to give an example: each of the three testing sessions performed in front of the pair 4 *vs* 3 dots bear the same test identifier (ID) ‘1’; each of the three sessions performed in front of 4 *vs* 5 dots bear the same ID ‘2’, etc.). A glmer function with a binomial distribution as error structure presented overdispersion values > 5, which forced to fall back on a model using a quasi-binomial error structure: `glmmPQL(cbind(score_even,score_odd) ~ treatment [or relation], random= ~1|ID, family= quasibinomial, data.`

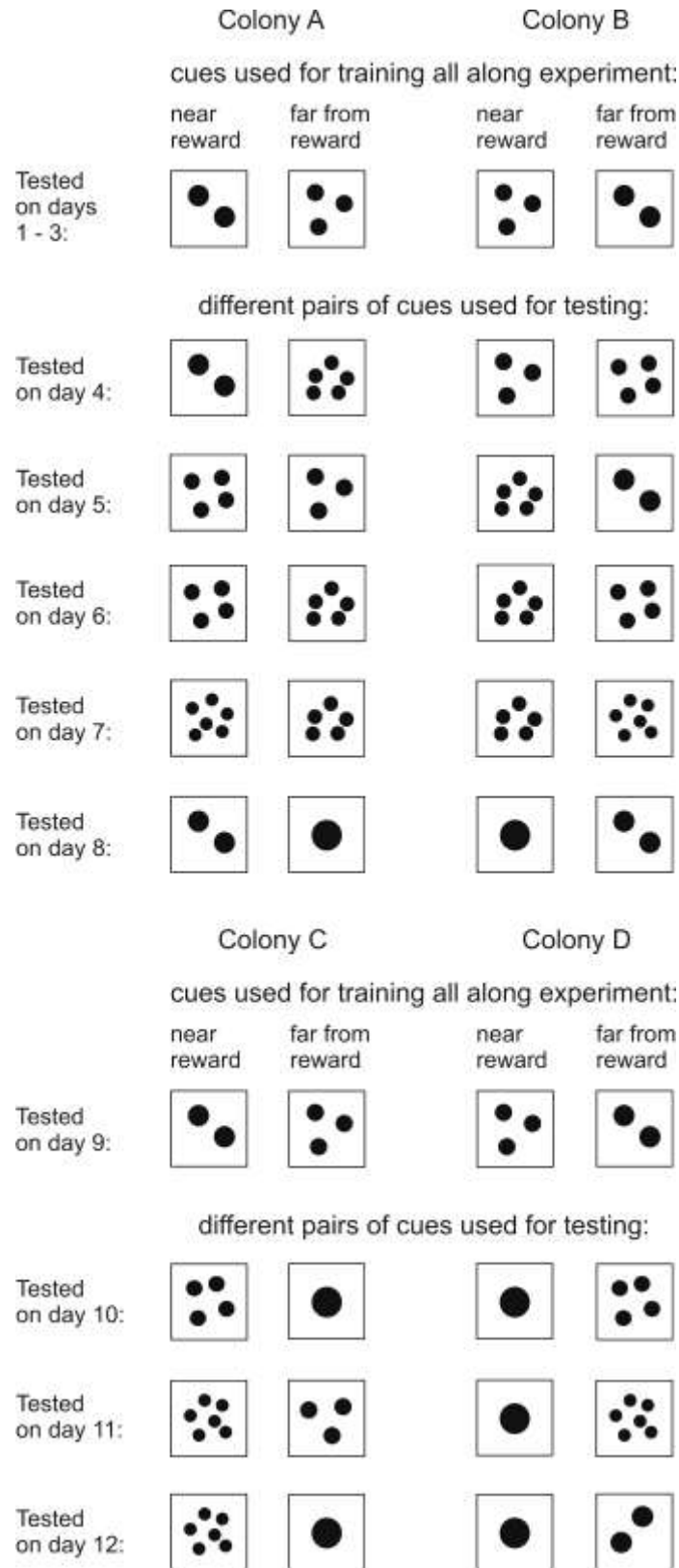


Figure 2. Pairs of cues presented, according to the daily experimental schedule, to 10 young ants from each colony, while these colonies continued, all along the experiment, the same training as during the first days

The cue corresponding to the correct response is shown on the left side of each presented pair, although this parity may be presented to the ants on the left or the right-side during testing.

### 3. Results

Young ants of colonies A and B underwent preliminary testing during the days 1 to 3 to ensure that they duly learned the cue having the parity they were expected to choose during the following days when in presence of cues showing different number of dots. Table 1 shows that it was already the case after 12 hours of training. As the preliminary tests made on colonies C and D provided a high correct score already after 8 hours of training, 24 hours of preliminary training were for them considered sufficient to avoid any doubts as for the success of their training.

Table 1. Young ants' visits to a 'correct', even or odd, number *versus* a 'wrong', odd or even, number, the 'correct' number being the one (marked with a \*) having the same parity as the number to which they were trained

| Day  | Time | Colony A     |          |           | Colony B     |          |           |
|--|------|--------------|----------|-----------|--------------|----------|-----------|
| Preliminary testing sessions examining the effectiveness of learning |      |              |          |           |              |          |           |
|  |      | In front of  | score    | % correct | In front of  | score    | % correct |
| 1  | 12 h | 2* vs 3 dots | 30 vs 10 | 75.0%     | 3* vs 2 dots | 33 vs 20 | 62.6%     |
|  | 24 h |              | 35 vs 16 | 68.6%     |              | 32 vs 11 | 74.4%     |
| 2  | 36 h |              | 28 vs 5  | 84.8%     |              | 24 vs 7  | 77.4%     |
|  | 48 h |              | 38 vs 2  | 95.0%     |              | 34 vs 11 | 75.5%     |
| 3  | 60 h |              | 34 vs 5  | 87.2%     |              | 36 vs 7  | 83.7%     |
|  | 72 h |              | 26 vs 3  | 89.6%     |              | 28 vs 4  | 87.5%     |
| Testing in front of pairs differing from those seen during learning  |      |              |          |           |              |          |           |
| 4  | 8 h  | 2* vs 5 dots | 34 vs 3  | 91.9%     | 3* vs 4 dots | 33 vs 7  | 82.5%     |
|  | 16 h |              | 42 vs 3  | 93.3%     |              | 34 vs 0  | 100.0%    |
|  | 24 h |              | 32 vs 5  | 86.5%     |              | 21 vs 3  | 87.5%     |
| 5  | 8 h  | 4* vs 3 dots | 49 vs 8  | 85.9%     | 5* vs 2 dots | 34 vs 6  | 85.0%     |
|  | 16 h |              | 45 vs 7  | 86.5%     |              | 20 vs 5  | 80.0%     |
|  | 24 h |              | 29 vs 7  | 80.6%     |              | 23 vs 10 | 69.7%     |
| 6  | 8 h  | 4* vs 5 dots | 33 vs 8  | 80.5%     | 5* vs 4 dots | 33 vs 7  | 82.5%     |
|  | 16 h |              | 22 vs 8  | 73.3%     |              | 29 vs 10 | 74.4%     |
|  | 24 h |              | 21 vs 10 | 67.7%     |              | 20 vs 10 | 66.7%     |
| 7  | 8 h  | 6* vs 5 dots | 23 vs 21 | 52.3%     | 5* vs 6 dots | 18 vs 21 | 46.1%     |
|  | 16 h |              | 17 vs 16 | 51.5%     |              | 12 vs 17 | 41.4%     |
|  | 24 h |              | 18 vs 12 | 60.0%     |              | 15 vs 17 | 46.9%     |
| 8  | 8 h  | 2* vs 1 dot  | 27 vs 4  | 87.1%     | 1* vs 2 dots | 26 vs 5  | 83.9%     |
|  | 16 h |              | 41 vs 7  | 85.4%     |              | 33 vs 8  | 80.5%     |
|  | 24 h |              | 28 vs 9  | 75.7%     |              | 26 vs 6  | 68.3%     |
| Day  | Time | Colony C     |          |           | Colony D     |          |           |
| Preliminary testing sessions examining the effectiveness of learning |      |              |          |           |              |          |           |
|  |      | In front of  | score    | % correct | In front of  | score    | % correct |
| 9  | 8 h  | 2* vs 3 dots | 36 vs 7  | 83.7%     | 3* vs 2 dots | 25 vs 7  | 78.1%     |
|  | 16 h |              | 41 vs 8  | 83.7%     |              | 25 vs 7  | 78.1%     |
|  | 24 h |              | 28 vs 7  | 80.0%     |              | 20 vs 6  | 76.9%     |
| Testing in front of pairs differing from those seen during learning  |      |              |          |           |              |          |           |
| 10   | 8 h  | 4* vs 1 dot  | 25 vs 10 | 71.4%     | 1* vs 4 dots | 25 vs 10 | 71.4%     |
|  | 16 h |              | 44 vs 2  | 95.6%     |              | 20 vs 7  | 74.1%     |
|  | 24 h |              | 31 vs 5  | 86.1%     |              | 22 vs 8  | 73.3%     |
| 11   | 8 h  | 6* vs 3 dots | 32 vs 0  | 100.0%    | 1* vs 6 dots | 28 vs 9  | 75.7%     |
|  | 16 h |              | 20 vs 1  | 95.2%     |              | 19 vs 11 | 63.3%     |
|  | 24 h |              | 20 vs 5  | 80.0%     |              | 20 vs 7  | 74.1%     |
| 12   | 8 h  | 6* vs 1 dot  | 42 vs 3  | 93.3%     | 1* vs 2 dots | 20 vs 11 | 64.5%     |
|  | 16 h |              | 20 vs 4  | 83.3%     |              | 21 vs 8  | 72.4%     |
|  | 24 h |              | 20 vs 5  | 80.0%     |              | 20 vs 9  | 69.0%     |

The table gives the numbers of correct *versus* wrong responses of young ants of colonies A, B, C and D, as well as the % of correct responses, in the presence of several pairs of even and odd numbers. In front of 5 and 6 dots the ants could no longer discriminate the correct parity, essentially when it was the odd number. The young ants' sensitivity to parity exists thus from the start of the numerosity (number 1) until at least the number 6.

A look at Table 1 shows that during tests in which the ants were presented with a pair of cues, each bearing a number of dots varying from 1 to 6, and each pair being distinct from that present in the foraging area during learning, they chose the cue bearing a number with the same parity as the learned number. The exception holds for the pair of 5 vs 6 dots, for

which the comparison of these two numbers of dots was found to have exceeded the capacity of the ants' number discrimination. The results concerning this outlying pair tested on day 7 shall be detailed separately and are not taken into account in an inferential analysis aimed at measuring the response of young workers to a choice of parity within their range of number discrimination capability. This statistical analysis shall also not include the tests that were performed on pairs of cues in which one of the cues bore the same number as that of the learned one, as in the pairs 2 vs 5, 2 vs 1 and 3 vs 4 (i.e., the tests performed on day 4 with colonies A and B and on day 8 with only colony A). In front of such pairs, the correct choice of the ants could have resulted from something else than a counting process, although the latter cannot be excluded.

A parametric ANOVA on the 33 tests thus finally retained (named 'definitive tests') and the 18 preliminary ones assessing learning ( $N = 51$ ) showed no significant difference between the scores of the correct choices of the different colonies, except for colony D whose definitive tests had a lower score (on average 70.9%) than those of the other colonies (whose average scores ranged from 77.2% to 87.2%). The P values obtained with or without the definitive tests performed on colony D were 0.004 and 0.17. The use of a parametric ANOVA on the preliminary tests assessing learning is validated by the homogeneity of the variances (Levene's test:  $P = 0.20$ ) and the normality of the distribution of residuals (Henry's line) This is also the case, but to a lesser extent, for the definitive tests (Levene's test:  $P = 0.091$ ). In a learning process, the responses follow a Gaussian distribution when they are issued from a successful learning (see Cammaerts & Cammaerts, 2022 and 2023 for more explanation). The responses of the 18 tests that were done to verify the success of learning had a mean of 80.21%, a median of 79.71% and a variance amounting to 60,39%, what can agree with a Gaussian distribution. However, the responses to the 33 retained definitive tests had a mean of 78.83%, a median of 80% and a variance of 83,52%. Without colony D, these values were respectively 81.81%; 80.90% and 75.45%, suggesting that, in addition to reacting to learning, some of the responses from colony D were made randomly.

Overall, the young ants choose the right parity in all of the 33 definitive tests (as well as, with the exception of the tests made on day 7, when all the 42 tests not intended for checking learning were taken into account, i.e., those made at days 4 to 6, 8 and 10 to 12) whether the number of dots on the learned cue (the 'correct' parity) was larger or smaller than that of the parity that was not to be learned (the 'wrong' parity). The % of correct responses observed during the 33 trials varied from 63.3 to 100% (Table 1) with an average of 78.8%, and little variation in the responses (95% confidence limits: 75.6 and 82.1%). The % of 'wrong' choices varied thus from 0 to 36.7%. It may therefore seem superfluous to carry out a Wilcoxon test on the correct and wrong responses of the pairs of cues presented ( $P < 10E-6$  for  $n = 33$ ).

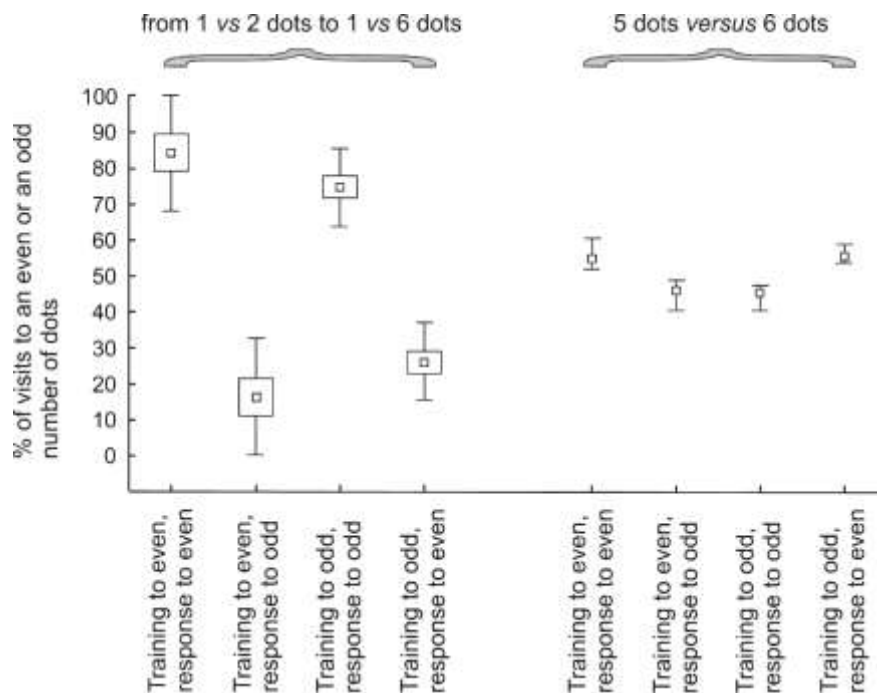


Figure 3. Graphical summary of the results of the 33 retained tests (left side) and of the 6 tests on pairs comparing 5 and 6 dots (right side), performed to determine if young ants recognize the parity of numbers different from the learned one. Mean, 95% confidence interval and extremes of the ants' responses.

Figure 3 graphically summarizes the mean and variability of the responses of the ants to the ‘correct’ and ‘wrong’ presented cues after having been trained to learn a number with an even (i.e., 2) or an odd (i.e., 3) parity. It tells us that the responses clearly showed a positive reaction towards the parity that was learned and a negative reaction towards the parity that was not learned. It shows also that when having been trained to an even number, the mean difference between correct and wrong responses was higher than when having been trained to an odd number. Indeed, trained to even, the mean score to even was 83.94% while that to odd was 16.02% and trained to odd, the mean score to odd was 74.53% while that to even was 25.46%. In other words, the difference (the distance) between correct and wrong mean score was higher (67.92%) when trained to an even number than when trained to an odd number (49.07%).

As for their choice between 5 and 6 dots, the young ants of colonies A and B could not readily discriminate between these numbers, whenever the learned parity was even or odd, showing the limit of the numbers’ parity discrimination by the young workers of the ant *M. sabuleti* (based on the total number of correct and wrong responses, a binomial test gives respectively  $P = 0.22$  and  $0.18$  and a Chi-square test:  $\chi^2 = 1.40$ ,  $df = 1$ ,  $0.10 < P < 0.25$ ).

It should also be noted that for the 33 retained definitive tests, the young ants on average achieved a higher score when they reacted to an even number having learned an even one than when they reacted to an odd number having learned an odd one (mean values: 83.94% and 74.53%; ANOVA:  $P = 0.0018$ ). Such a significant difference between correct scores for even and odd numbers when trained to the corresponding parity was not found when accomplished foragers, thus much older workers, were tested (Cammaerts & Cammaerts 2023, ANOVA:  $P = 0.22$ , hence, this non-significant result was not reported in the preceding paper, but can be seen in its Figure 3, where the mean scores amounted 85.15% against 87.22%).

In front of 5 vs 6 dots, thus at the limit of their discrimination capability, and insofar as only two groups, each with three tests, could be compared with each other, the young ants also responded better to the even number when learned an even one than to the odd number when learned an odd one (respectively meanly 54.21% and 45.00%; ANOVA:  $P = 0.0381$ ). However, the reverse was observed in older ants, where in front of 7 vs 8 dots, the limit of discrimination capability, the old foragers responded significantly better to the odd number when learned an odd one than to the even number when learned an even one (Cammaerts & Cammaerts, 2023: mean score 58.22% against 44.87%; ANOVA:  $P < 10E-5$ ).

A GLMM analysis with ‘treatment’ (i.e., learned parity) as the only fixed effect showed of course that this predictor was highly significant. The mean level of the proportions of ants sighted near the even number of dots of a pair was drastically lower when the learned parity was odd ( $P = 0$ ). Another way of looking at it was to raise the logit value (-2.79) of the coefficient estimate of the output as a power of  $e$ : it showed that the odds of responding to an even number were only 0.061 times the odds of responding to an odd number when the learned parity was odd. Complementarily, when the learned cue was even, the proportions of ants near an odd number were negligible. The interest of a GLMM analysis remains then in the effect of the relations between the numbers of elements of the two cues presented to the ants.

When the numerical relations between the cues of the presented pairs were calculated with the numbers of dots ordered as the learned parity (i.e., the one to which the ants were expected to respond, these cues being marked with an asterisk in Table 1 and in the Appendix), no any value of the predictors ‘pair’ of numbers, ‘ratio’ and ‘difference divided by the mean’ of the two presented numbers was significant at the  $\alpha = 0.05$  level, but the values of the predictors ‘ratio’ corresponding to the pairs 4\* vs 1, 4\* vs 3, 4\* vs 5, 6\* vs 1, 6\* vs 3 and the values of ‘difference between the two numbers of dots divided by their mean’ corresponding to the pairs 1\* vs 2, 1\* vs 4, 1\* vs 6, 5\* vs 2 and 5\* vs 4 were all at the significance level  $0.095 < P < 0.125$ , i.e., much lower than that of the other pairs ( $0.25 < P < 0.88$ ). It nevertheless showed that when having been trained to an even (i.e., 2) number of dots, and tested in front of a pair of cues, one of which bears an even number of dots, the mean level of the ants’ response to an even number was positively influenced by the sight of the even number. By raising the coefficient estimates of the outputs of these predictors (these logit values varied from 1.98 to 3.61) as a power of  $e$ , the odds of responding to an even number when having learned another even number were 7.25 to 29.78 times the odds of responding to an odd number, what was less than what was observed in older, forager, ants (49 to 151 times: Cammaerts & Cammaerts, 2023).

In the same way, when having been trained to an odd (i.e., 3) number of dots, and tested in the presence of a pair of cues, one of which carried an even number of dots, the mean level of the ants’ response to an even number was negatively influenced by the sight of the odd number. By exponentiating the coefficient estimates of the outputs of these predictors (these logit values varied from -2.37 to -1.98), the odds of responding to an even number when having learned another even number were 0.09 to 0.14 times the odds of responding to an odd number, what was slightly more than what was observed in older, forager, ants (0.02 to 0.05: Cammaerts & Cammaerts, 2023).

To allow a comparison with older ants in which the values of the same predictors were all highly significant (Cammaerts & Cammaerts, 2023), the present Appendix shows the results for the predictors 'ratio' and 'difference on mean'.

#### 4. Discussion

Having previously shown that elderly *M. sabuleti* forager ants can recognize the even and odd character of numbers of dots from 1 to 7 (Cammaerts & Cammaerts, 2023) and that they possess, early in their life (possibly natively), a number line (Cammaerts & Cammaerts, 2020b), although they have to acquire the notion of zero through experience (Cammaerts & Cammaerts, 2020c), we aimed to examine whether these ants' ability to recognize the parity of numbers was innate or acquired through experience.

Our results showed that young workers (nurses), in all likelihood no older than a month and a half (see below), settled in small single-cohort colonies, forced to forage and learned, by operant conditioning, to recognize either 2 or 3 dots displayed on a stand, were, when tested in front of two stands differing from each other in the number of dots and their parity, more responsive to the stand bearing the same parity as the one learned. They thus recognized the parity corresponding to the one they learned to associate with a reward. They were so able to recognize the parity of numbers from 1 to at least 6. It should be noted that no attempt was made to test the response of ants that would be put in front of a stand bearing a higher number of dots, next to a stand bearing a much smaller number, with therefore a large numerical difference between the two cues, such as between 2 and 7 or 1 and 8. As for their ability to discriminate close numbers, it reached the comparison of 4 and 5, which is less than that of older ants, who can differentiate until 6 from 7. Furthermore, these young ants responded more to a number corresponding to the learned parity when it was even than when it was odd, whereas older ants (experienced foragers: Cammaerts & Cammaerts, 2023) responded correctly and equally to either kind of learned parity.

The sensitivity of *M. sabuleti* ants to the parity of numbers appears therefore to be already present at an early stage of their worker life, although this apparently innate ability is incomplete and will be perfected later on when they will acquire the status of experienced foragers. A precocious maturity, such as that possessed here by young workers that were forced to act as older ones, however, may prevent them from being as efficient as experienced foragers. As a dramatic example, honeybee workers prematurely foraging in response to environmental stressors perform and survive poorly outside the hive. Modeling showed that with higher mortality and less food collected, more young worker bees entered an immature foraging stage, dying soon afterwards and leaving the hive with abandoned brood and a shortage of food, which will trigger its rapid decline (Perry, S øvik, Myerscough & Barron, 2015).

It is not known at what age *M. sabuleti* workers, not subjected to forced conditions, would have left the nest to forage and, having reached this behavioral stage, to what extent the relevant behavioral maturity is simply age-related or, in addition, is acquired through experience. Basic studies on ant polyethism were nevertheless carried out long ago on two closely related *Myrmica* species.

In *M. scabrinodis*, the workers' locomotor activity and cuticular pigmentation change during their aging from the stage of nurses, situated in the core of the nest, to foragers present outside the nest, through an intermediate community of workers named 'domestics' (Weir, 1958) or 'cleaners' (Mersch, Crespi & Keller, 2013) present at an intermediate topological location. In *M. rubra* workers, Cammaerts-Tricot (1974) could easily distinguish five cuticular pigmentation degrees and relate them with the topological position and activity of the ant in and outside the nest, as well as, under laboratory conditions, with the age of the ant from reared pupae. Under laboratory conditions (constant temperature, humidity and photoperiod), pupae gave birth to very pale callows (belonging to the light yellow-brown pigmentary degree 1 where only the eyes are dark) that became slightly darkened (degree 2) in about three or four days. They reached a more pigmented degree 3 after sixteen or eighteen days, when the back of head and gaster turned brown, and reached a pigmentation degree 4 after three or four more months, when the dorsal surface of thorax also appeared brown. Finally, they reached the darkest pigmentation degree 5 after at least one or two more months, thus in a total of about 6 months (Cammaerts-Tricot, 1974). Workers with pigmentation stages 4 and especially 5 are known to be accomplished foragers (M.-C. Cammaerts, unpublished observations).

In the wild, in colonies of *M. rubra* with a natural demographic structure and submitted to climatic constraints such as those prevailing in the vicinity of Brussels, egg-laying, larval and nymphal development are fairly continuous throughout the year, but with two peaks, starting with births at the beginning and at the end of the warm season. Accordingly, in a natural nest, two groups of ants of different ages exist throughout the year. A first group of workers emerge from pupae at the beginning of the warm season and show pigmentation degree 2 in July or August, reach pigmentation degree 3 one or two months later, in September or October, overwinter and reach degree 4 in next July or August until reaching degree 5 in December. They then hibernate again, dying in July or August after having maximally reached the age of about 2 years and one or two months. A second group of workers is issued from pupae at the end of



the warm season and reach pigmentation degree 2 in October or November, degree 3 in January and degree 4 in July or August, pigmentation degree 5 in December or January and finally, die during the next warm season after having lived maximally 2 years (Cammaerts-Tricot, 1977).

Similar to what happens in honeybees (Huang & Robinson, 1996; Leoncini, Crauser, Robinson & Le Conte, 2004), task performance in *M. sabuleti* ants appears not to be rigidly determined by age, but regulated according to the needs of the society. It explains why the young ants that were working as nurses and were taken from the core of the nest to form single-cohort colonies of ants aged of about one month or so, took over food harvesting at an early stage of their life. We estimate that, after they were placed in single-cohort colonies, about one quarter of these young workers had already started foraging when we picked up the required number of individuals for testing.

In the ant species *Pogonomyrmex californicus*, when the lightest, thus the youngest workers of the society were taken out of a field colony and isolated as a single cohort in a laboratory nest, they initiate their transition to foragers 100 days earlier than at age-typical, control colonies. Their juvenile and ecdysteroid hormone contents were not related to their age, but to their transition from inside to outside activities (Dolezal, Brent, Hölldobler & Amdam, 2012).

By individually tracking tagged *Camponotus fellah* ants during 5 months (Richardson *et al.*, 2021; see also a commented summary in Crall, 2021), it was shown that the transition from a young worker (a core nurse) to an older one (an accomplished forager), thus from a state of low-maturity to high-maturity behavior, was sigmoidal, the change being abrupt at the inflection point and lasting in total some 40 days. However, the individual timing of this change is variable, stochastic i.e., independent of the age of the individual. In this ant species, the first trip outside the nest occurred  $44 \pm 28$  days (mean and standard error) after birth ( $n = 31$ ) thus with a large individual variation.

In honeybees, the behavioral transition from within-hive tasks to foraging occurs ca. at the age of 3 weeks and is linked to a rise in juvenile hormone and a reorganization of the mushroom bodies of the protocerebrum, a brain region associated with learning and memory. However, this natural behavioral maturation is flexible and these hormonal and structural changes in the brain occur also when bees are driven to forage as early as 4 days of age when the survival of the colony needs it (Fahrbach & Robinson, 1996). After a first, but age-related maturational phase, nurse bees aged from 8 days can transition to further behavioral stages or, if necessary, remain nurses during some more weeks, the timing of this transition being driven by social and physiological factors on any given day. Four genes may be involved in this behavioral transition (Whitfield *et al.*, 2006).

It must be noted that not all social insects show behavioral flexibility. In the ant *Pogonomyrmex badius*, removing half of the forager population does not stimulate precocious foraging in younger ants with, as a consequence, larvae die in proportion of the foragers removed (Kwapich & Tschinkel, 2015).

Molecular basis for transition between behavioral states is the object of recent researches in social insects and a promise for further comprehension of these changes. For example, in the ant *Pheidole pallidula*, minor workers perform foraging while major specialize in defense, but the latter can switch to foraging tasks according to the needs of the colony. A foraging gene *ppfor* encodes a cGMP-dependent protein kinase (PKG), more expressed in major than in minor workers. Activation of PKG increased defense and reduced foraging behavior, especially in the major caste, which showed thus to be more behaviorally flexible than the minor caste (Lucas & Sokolowski, 2009). In the fire ant *Solenopsis invicta*, knocking down the expression of the foraging gene *Sifor* in forager workers triggered their behavioral transition toward nurses, while activating PKG produced a transition from nurse behavior to that of a forager (Chen *et al.*, 2022).

In the same way as for older ants (Cammaerts & Cammaerts, 2023), distinguishing parity of numbers does not mean that young ants know what an even or odd quantity is. Their judgment may simply depend on a feature of the visual aspect of the quantity seen, such as the symmetry or asymmetry of the image. By a learning process, honeybees can acquire a preference for symmetrical or asymmetrical visual patterns (Giurfa, Eichmann & Menzel, 1996). Learning could perhaps explain why, in the course of aging, the apparently innate preference of young *M. sabuleti* ants for even parity can become matched by their response to odd parity.

What we have found in young *M. sabuleti* ants as for their parity sensitivity, as for their possession of a number line (Cammaerts & Cammaerts, 2020b) and as for their acquisition of the notion of zero (Cammaerts & Cammaerts, 2020c) is rather similar to what exists in humans' children 1 to 3 years old. The latter have a number line, acquire the notion of zero, distinguish even and odd numbers, but have to finalize this last skill at the age of more than eight (Berch, Foley, Hill & Ryan, 1999). In non-human mammals, contrary to what is known in ants, behavioral maturation is strongly age-dependent (e.g., Pusey 1990 on chimpanzees).

To conclude, young ants, of an age corresponding to that of nurses, forced to behave as older foragers, were sensitive to the parity of numbers (quantities) with a preference for a parity corresponding to the parity of a learned, although

different number. They thus possess this skill early in life, possibly innately. They achieved on average a higher score when they reacted to an even number having learned even than when they reacted to an odd number having learned odd and their visual discrimination of close numbers was found to be limited to 4 versus 5 elements (black dots). When they will grow older, becoming accomplished foragers, their correct response to even and odd numbers will become more balanced and their visual discrimination limit for close numbers will be 6 versus 7 elements. Their performance therefore increases with age during their forager life, but it is not known whether this is due to an age-related maturation process or to acquired experience. Being able to recognize even and odd quantities may provide ants with useful information for finding their way in their natural environment, filled of numerous visual cues, that can be recognized and memorized when foraging and returning to the nest.

## References

- Berch, D. B., Foley, E. J., Hill, R. J., & Ryan, P. M. (1999). Extracting parity and magnitude from Arabic numerals: developmental changes in number processing and mental representation. *Journal of Experimental Child Psychology*, 74(4), 286-308. <https://doi.org/10.1006/jecp.1999.2518>
- Bernard, J., Stach, S., & Giurfa, M. (2006). Categorization of visual stimuli in the honeybee *Apis mellifera*. *Animal Cognition*, 9(4), 257-270. <https://doi.org/10.1007/s10071-006-0032-9>
- Cammaerts, M.-C., & Cammaerts R. (2019). Ants are at the first stage of the notion of zero. *International Journal of Biology*, 11(1), 54-65. <https://doi.org/10.5539/ijb.v11n1p54>
- Cammaerts, M.-C., & Cammaerts, R. (2020b). Young ants already possess a mental number line. *International Journal of Biology*, 12(2), 1-12. <https://doi.org/10.5539/ijb.v12n2p1>
- Cammaerts, M.-C., & Cammaerts, R. (2020c). Ants acquire the notion of zero through experiences. *International Journal of Biology*, 12(2), 13-25. <https://doi.org/10.5539/ijb.v12n2p13>
- Cammaerts, M.-C., & Cammaerts, R. (2022). Association between visual cues and time of day in an ant. *Journal of Ethology*, 40, 223-232. <https://doi.org/10.1007/s10164-022-00751-4>
- Cammaerts, R., & Cammaerts, M.-C. (2020a). Ant's mental positioning of amounts on a number line. *International Journal of Biology*, 12(1), 30-45. <https://doi.org/10.5539/ijb.v12n1p30>
- Cammaerts, R., & Cammaerts, M.-C. (2023). Capability of workers of the ant *Myrmica sabuleti* to categorize numbers of elements into even and odd. *International Journal of Biology*, 15(1), 45-57. <https://doi.org/10.5539/ijb.v15n1p45>
- Cammaerts-Tricot, M.-C. (1974). Production and perception of attractive pheromones by differently aged workers of *Myrmica rubra* (Hymenoptera Formicidae). *Insectes Sociaux*, 21(3), 235-247. <https://doi.org/10.1007/BF02226916>
- Cammaerts-Tricot, M.-C. (1977). Etude démographique annuelle des sociétés de *Myrmica rubra* L. des environs de Bruxelles. *Insectes Sociaux*, 24(2), 147-161. <https://doi.org/10.1007/BF02227168>
- Chen, J., Zhou, Y., Lei, Y., Shi, Q., Qi, G., He, Y., & Lyu, L. (2022). Role of the *foraging* gene in worker behavioral transition in the red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae). *Pest Management Science*, 78(4). <https://doi.org/10.1002/ps.6921>
- Crall, J. (2021). Social Insects: stochastic switches and behavioral maturation in ants. *Current Biology*, 31(10), R481-R483. <https://doi.org/10.1016/j.cub.2021.03.076>
- Dehaene, S., Bossini, S., & Giraux, P. (1993). The mental representation of parity and number magnitude. *Journal of Experimental Psychology: General*, 122(3), 371-396. <https://doi.org/10.1037/0096-3445.122.3.371>
- Dolezal, A. G., Brent, C. S., Hölldobler, B., & Amdam, G. V. (2012). Worker division of labor and endocrine physiology are associated in the harvester ant, *Pogonomyrmex californicus*. *Journal of Experimental Biology*, 215, 454-460. <https://doi.org/10.1242/jeb.060822>
- Fahrbach, S. E., & Robinson, G. E. (1996). Juvenile hormone, behavioral maturation, and brain structure in the honey bee. *Developmental Neuroscience*, 18(1-2), 102-114. <https://doi.org/10.1159/000111474>
- Giurfa, M., Eichmann, B., & Menzel, R. (1996). Symmetry perception in an insect. *Nature*, 382, 458-461. <https://doi.org/10.1038/382458a0>
- Hines, T. M. (1990). An odd effect: Lengthened reaction times for judgments about odd digits. *Memory & Cognition*, 18, 40-46. <https://doi.org/10.3758/BF03202644>
- Howard, S. R., Greentree, J., Avarguès-Weber, A., Garcia, J. E., Greentree, A. D., & Dyer, A. G. (2022). Numerosity categorization by parity in an insect and simple neural network. *Frontiers in Ecology and Evolution*, 10, 805385. <https://doi.org/10.3389/fevo.2022.805385>

- Huang, Z-Y., & Robinson, G. E. (1996). Regulation of honey bee division of labor by colony age demography. *Behavioral Ecology and Sociobiology*, *39*, 147-158. <https://doi.org/10.1007/s002650050276>
- Kwapich, C., & Tschinkel, W. R. (2015). Limited flexibility and unusual longevity shape forager allocation in the Florida harvester ant (*Pogonomyrmex badius*). *Behavioral Ecology and Sociobiology*, *70*, 221-235. <https://doi.org/10.1007/s00265-015-2039-1>
- Leoncini, I., Crauser, D., Robinson, G. E., & Le Conte, Y. (2004). Worker-worker inhibition of honey bee behavioural development independent of queen and brood. *Insectes Sociaux*, *51*, 392-394. <https://doi.org/10.1007/s00040-004-0757-x>
- Lucas, C., & Sokolowski, M. B. (2009). Molecular basis for changes in behavioral state in ant social behaviors. *PNAS*, *106*(15), 6351-6356. <https://doi.org/10.1073/pnas.0809463106>
- Mersch, D. P., Crespi, A., & Keller, L. (2013). Tracking individuals shows spatial fidelity is a key regulator of ant social organization. *Science*, *340*, 1090-1093. <https://doi.org/10.1126/science.1234316>
- Miller, K., & Gelman, R. (1983). The child's representation of number: A multidimensional scaling analysis. *Child Development*, *54*, 1470-1479. <https://doi.org/10.2307/1129809>
- Perry, C. J., Søyvik, E., Myerscough, M. R., & Barron, A. B. (2015). Rapid behavioral maturation accelerates failure of stressed honey bee colonies. *PNAS*, *112*(11), 3427-3432. <https://doi.org/10.1073/pnas.1610243113>
- Pusey, A. E. (1990). Behavioural changes at adolescence in chimpanzees. *Behaviour*, *115*(3-4), 203-2465. <https://doi.org/10.1163/156853990X00581>
- R Core Team (2021). R: A language and environment for statistical computing. Vienna: The R Foundation for Statistical Computing.
- Reynvoet, B., & Brysbaert, M. (1999). Single-digit and two-digit Arabic numerals address the same semantic number line. *Cognition*, *72*(2), 191-201. [https://doi.org/10.1016/S0010-0277\(99\)00048-7](https://doi.org/10.1016/S0010-0277(99)00048-7)
- Richardson, T. O., Kay, T., Braunschweig, R., Journeau, O. A., Rüegg, M., McGregor, S., ... Kemmer, L. (2021). Ant behavioral maturation is mediated by a stochastic transition between two fundamental states. *Current Biology*, *31*(10), 2253-2260. <https://doi.org/10.1016/j.cub.2020.05.038>
- Weir, J. S. (1958). Polyethism in workers of the ant *Myrmica*. *Insectes Sociaux*, *54*, 97-128. <https://doi.org/10.1007/BF02222431>
- Whitfield, C. W., Ben-Shahar, Y., Brillet, C., Leoncini I., Crauser, D., Le Conte, Y., ... Robinson, G. E. (2006). Genomic dissection of behavioral maturation in the honey bee. *Biological Science*, *103*(44), 16068-16075. <https://doi.org/10.1073/pnas.0606909103>
- Zazkis, R. (1998). Odds and ends of odds and evens: An inquiry into students' understanding of even and odd numbers. *Educational Studies in Mathematics*, *36*, 73-89. <https://doi.org/10.1023/A:1003149901409>

**Appendix**

Results of GLMM analyses with as sole predictor the ratio of the two presented numbers or their difference on the mean, the calculation being done by prioritizing the number corresponding to the learned parity. The first column gives the numerical value of the predictor and the last column its correspondence with the pair of dots presented to the ants. An \* marks the cue bearing the parity corresponding to the learned one. 33 testing sessions were taken into account. Pairs with the best response to the parity of the learned number (i.e., 2 or 3), and the associated probability, are written in bold.

Function: glmmPQL(cbind(score\_even, score\_odd) ~ ratio, random=~1|ID, family=quasibinomial,data)

|                 | Value     | Std.Error | DF | t-value   | p-value       | cue* vs cue    |
|-----------------|-----------|-----------|----|-----------|---------------|----------------|
| (Intercept)     | -0.908856 | 0.2786849 | 22 | -3.261231 | 0.0036        |                |
| cues_ratio 0.25 | -0.076961 | 0.3996988 | 1  | -0.192548 | 0.8789        | 1* vs 4        |
| cues_ratio 0.5  | -0.224603 | 0.3459819 | 1  | -0.649176 | 0.6334        | 1* vs 2        |
| cues_ratio 0.8  | 1.981493  | 0.3934691 | 1  | 5.035955  | <b>0.1248</b> | <b>4* vs 5</b> |
| cues_ratio 1.25 | -0.202027 | 0.3889085 | 1  | -0.519471 | 0.6950        | 5* vs 4        |
| cues_ratio 1.33 | 2.629998  | 0.3971841 | 1  | 6.621609  | <b>0.0954</b> | <b>4* vs 3</b> |
| cues_ratio 2    | 3.393762  | 0.5895176 | 1  | 5.756846  | <b>0.1095</b> | <b>6* vs 3</b> |
| cues_ratio 2.5  | -0.390427 | 0.4101826 | 1  | -0.951838 | 0.5157        | 5* vs 2        |
| cues_ratio 4    | 2.680813  | 0.4248905 | 1  | 6.309420  | <b>0.1001</b> | <b>4* vs 1</b> |
| cues_ratio 6    | 2.830668  | 0.4695171 | 1  | 6.028894  | <b>0.1046</b> | <b>6* vs 1</b> |

Function: glmmPQL(cbind(score\_even, score\_odd) ~ relative diff., random=~1|ID, family=quasibinomial,data)

|                          | Value      | Std.Error | DF | t-value   | p-value       | cue* vs cue    |
|--------------------------|------------|-----------|----|-----------|---------------|----------------|
| (Intercept)              | 1.0726368  | 0.2777637 | 22 | 3.861689  | 0.0008        |                |
| cues_diff_on_mean -0.667 | -2.2060958 | 0.3452403 | 1  | -6.390030 | <b>0.0988</b> | <b>1* vs 2</b> |
| cues_diff_on_mean -1.2   | -2.0584536 | 0.3990570 | 1  | -5.158294 | <b>0.1219</b> | <b>1* vs 4</b> |
| cues_diff_on_mean -1.429 | -1.9814926 | 0.3934691 | 1  | -5.035955 | <b>0.1248</b> | <b>1* vs 6</b> |
| cues_diff_on_mean 0.222  | -2.1835192 | 0.3882489 | 1  | -5.624020 | <b>0.1120</b> | <b>5* vs 4</b> |
| cues_diff_on_mean 0.286  | 0.6485051  | 0.3965382 | 1  | 1.635416  | 0.3494        | 4* vs 3        |
| cues_diff_on_mean 0.667  | 1.4122698  | 0.5890827 | 1  | 2.397405  | 0.2516        | 6* vs 3        |
| cues_diff_on_mean 0.857  | -2.3719198 | 0.4095572 | 1  | -5.791425 | <b>0.1089</b> | <b>5* vs 2</b> |
| cues_diff_on_mean 1.2    | 0.6993200  | 0.4242868 | 1  | 1.648225  | 0.3472        | 4* vs 1        |
| cues_diff_on_mean 1.429  | 0.8491758  | 0.4689708 | 1  | 1.810722  | 0.3212        | 6* vs 1        |

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