Differential effects of brain size on memory performance in parasitic wasps

van der Woude, E., Huigens, M. E., & Smid, H. M.

van der Woude, E., Huigens, M. E., & Smid, H. M. (2018). Differential effects of brain size on memory performance in parasitic wasps. Animal Behaviour, 141, 57-66. DOI: 10.1016/j.anbehav.2018.05.011

Differential effects of brain size on memory performance in parasitic wasps

Emma van der Woude[1*], Martinus E. Huigens[1,2], Hans M. Smid[1,3]

[1] Laboratory of Entomology, Wageningen University, Wageningen, the Netherlands

[2] Current address: Education Institute, Wageningen University, Wageningen, the Netherlands

[3] Corresponding author: Address: Hans M. Smid, Wageningen University, Laboratory of Entomology P.O. Box 16 - 6700 AA The Netherlands, email: hansm.smid@wur.nl, phone: +31317482791

Abstract

Small animals usually have relatively larger brains than large animals. This allometric brain-body size scaling is described by Haller's rule. However, one of the smallest insects on Earth, *Trichogramma evanescens* parasitic wasps show brain isometry, leading to similar relative brain sizes in small and large conspecifics. Somewhat larger *Nasonia vitripennis* parasitic wasps display diphasic brain-body size scaling with isometry in small individuals and allometry in large individuals. These two species may have undersized brains in small wasps, with reduced cognitive abilities. Here, we induced intraspecific body-size variation in genetically identical *T. evanescens* and *N. vitripennis* and examined cognitive trade-offs of brain scaling. We compared visual and olfactory memory retention between small and large conspecifics. Results show that diphasic brain scaling affects memory retention levels in *N. vitripennis*, whereas isometric brain scaling does not affect memory retention in *T. evanescens*. The two species may experience different evolutionary pressures that shaped the cognitive consequences of isometric brain-body size scaling. A possible trade-off of brain isometry in *T. evanescens* could be present in brain properties different from memory performance. In contrast, it may be more adaptive for *N. vitripennis* to invest in other aspects of brain performance, at the cost of memory retention.

Key words: Brain scaling, Haller's rule, isometry, learning, memory, parasitic wasp

Word count: 9038 including captions and references

Introduction

An individual's ability to learn and memorize has been related to the size of the brain, both absolute brain size and the size of the brain relative to total body size (Kotrschal et al., 2013; Roth & Dicke, 2005; Striedter, 2005). However, relative brain size also directly depends on body size: small animals have relatively larger brains than large animals. This is known as Haller's rule, and generally applies within and between animal species in all taxa (Gonda et al., 2011; Harvey & Krebs, 1990; Isler et al., 2008; Kruska, 1996; Pagel & Harvey, 1989; Rensch, 1948; Riveros & Gronenberg, 2010; Seid et al., 2011; Stuermer et al., 2003; Wehner et al., 2007). Haller's rule follows a power-law function in which the exponent, the scaling coefficient, determines the shape of the relationship. The more the scaling coefficient approaches 0, the stronger the negative allometry that Haller's rule describes. A scaling coefficient that equals 1 describes a linear relationship known as isometry.

Allometric brain-body size scaling may be a consequence of several different mechanisms through which neural architecture determines behavioural output (Willemet, 2013). For instance, those neurons involved in regulation of somatic processes may be lower in numbers and in complexity of their neurites when body size is small. However, for those neurons involved in cognitive processes, which is not necessarily different between small and large animals, the absolute, not relative, number and size of neurons and connections determines the required neural processing power (Chittka & Niven, 2009). Small animals may thus need to form relatively larger brains to achieve similar levels of cognition as large animals.

This allometry implies that small animals suffer high energetic costs, because brain tissue has a high metabolic rate (Aiello & Wheeler, 1995). These energetic costs can become too high to be overcome by the smallest animals, which limits body miniaturization (Eberhard & Wcislo, 2011). Interestingly, some of the smallest insect species show unique intraspecific brain scaling properties, possibly to avoid the energetic costs of having a relatively large brain (see Groothuis and Smid (2017) for a recent overview of brain scaling in differently-sized insects). An example is shown by a species of polymorphic leaf-cutter ants (*Atta colombica*), which vary in body length between 5–10 mm (Feener et al., 1988). Workers show an allometric relationship between brain and body size (Seid et al., 2011). However, a break point splits the allometry into two separate functions. Larger ants show a scaling coefficient of 0.29, which is comparable to scaling coefficients found for other ant species (Wehner et al., 2007). Smaller ants have a much larger scaling coefficient of 0.60.

Another example is shown by smaller *Nasonia vitripennis* parasitic wasps (Figure 1a). These wasps parasitize and develop inside fly puparia. Adult body size depends on the number of developing larvae inside the same host pupa through scramble competition, resulting in body lengths ranging between 1.2–2.4 mm measured from thorax to abdomen tip (Groothuis & Smid, 2017). Again, a break point divides

the wasps into two groups where the smallest wasps have a larger scaling coefficient, which in this case is similar to 1, resulting in isometric brain-body size scaling (Figure 2a).

The most extreme form of intraspecific brain scaling is shown by some of the smallest insects on Earth, *Trichogramma evanescens* parasitic wasps (Figure 1b). These minute wasps parasitize and develop inside lepidopteran eggs. Adult body size depends on scramble competition in a similar way as in *N. vitripennis*, resulting in body lengths ranging between 0.3–0.9 mm (Van der Woude & Smid, 2016; Van der Woude et al., 2013). *Trichogramma evanescens* wasps scale their brains isometrically with body size (Van der Woude et al., 2013; Figure 2a). Small and large individuals of this species have the same relative brain size, with brains that are much smaller in the smallest *T. evanescens* and much larger in the largest *T. evanescens* compared to species with allometric brain-body sized scaling.

The abovementioned examples show that isometric brain-body size scaling is observed in some of the smallest insects, possibly because small invertebrates avoid the excessive energetic costs that are associated with a relatively larger brain. A smaller relative brain size may enable smaller body sizes but may simultaneously cause trade-offs with brain performance when brains become too small to maintain all neural processing abilities. The smallest invertebrates could consequently show impaired learning abilities and reduced memory retention, and suffer more from the metabolic costs that are associated with forming and retaining long-term memory (Hoedjes et al., 2011; Margulies et al., 2005; Mery & Kawecki, 2005; Snell-Rood et al., 2009). Notably, intraspecific allometry reflects the more limited developmental plasticity of brain size compared to body size in response to environmental constraints such as resource availability. Isometric brain body-size scaling may require a higher level of developmental plasticity of brain tissues than allometric brain-body size scaling and such developmental plasticity may evolve under specific constraints that occur in the smallest insects.

In the present study, we examined cognitive trade-offs of the developmental processes that underlie isometric brain-body size scaling that result in a small brain. We compared memory performance (level and duration of memory retention) of small and large conspecifics of *T. evanescens* and *N. vitripennis* (Figure 1c), both after visual and olfactory conditioning. *Nasonia vitripennis* can form long-term memory of olfactory cues after a single experience of drilling a hole in the host pupa and feeding from its contents (Hoedjes & Smid, 2014; Schurmann et al., 2012). *Trichogramma evanescens* naturally hitchhike on mated female butterflies, which enables them to parasitize freshly laid eggs and form long-term memory of the butterfly's antiaphrodisiac pheromone (Huigens et al., 2009; Kruidhof et al., 2012). Associative learning of colours is less frequently studied in these species, but has been described in both (Keasar et al., 2000; Oliai & King, 2000).

We hypothesize that having a small brain compromises memory performance in small wasps of both species, because the observed isometry may be a result of a reduction in brain size beyond the size that is required to maintain the same brain performance as the larger conspecifics. We expect that these

effects are more pronounced in *T. evanescens* than in *N. vitripennis*, because brain isometry is observed over the full-size range in *T. evanescens*, which may be a consequence of more strongly miniaturized brains in small individuals than in *N. vitripennis*, where isometric-allometric brain body size scaling was observed.

Methods

Insects

Trichogramma evanescens Westwood (Hymenoptera: Trichogrammatidae) of inbred strain GD011 originating from a female collected near Wageningen, the Netherlands, were reared on UV-irradiated host eggs of Mediterranean flour moth *Ephestia kuehniella* (Lepidoptera: Pyralidae; obtained from Koppert Biological Systems, Berkel en Roderijs, The Netherlands) (Huigens et al., 2009; Van der Woude et al., 2013). The wasps were kept in a climate room (22±1°C, 50–70% rh, L16:D8), and used to create body-size variants as described below.

Nasonia vitripennis Walker (Hymenoptera: Pteromalidae) of inbred strain AsymCx, originating from a female collected in Leiden, the Netherlands (Breeuwer and Werren, 1995) were reared on *Calliphora vomitoria* pupae (obtained as maggots from Kreikamp, Hoevelaken, The Netherlands) in a climate cabinet (25±1°C, L16:D8) (Hoedjes et al., 2012). These pupae were also used as unconditioned stimulus in the conditioning assays of *N. vitripennis.*

Cabbage moths *Mamestra brassicae* (Lepidoptera: Noctuidae) were obtained from laboratory colony at Wageningen University, and were originally collected from cabbage fields near Wageningen. They were reared on cabbage plants (*Brassica oleracea*) in a climate room (21±2°C, 50–70% rh, L16:D8). Adult moths oviposited on sheets of filter paper, which were used as unconditioned stimulus in conditioning assays of *T. evanescens*. *Manduca sexta* (Lepidoptera: Sphingidae) hawkmoths (obtained as pupae from the Max Planck Institute for Chemical Ecology, Jena, Germany) were kept in a flight cage with tobacco plants (*Nicotiana tabacum* SR1) inside a climate cabinet (25±1°C, L16:D8) (Van der Woude & Smid, 2016). Eggs were harvested daily from this cage, stored at -20°C and used to rear small and large *T. evanescens*.

Induction of body size variation

We manipulated wasps to lay either large or small numbers of eggs inside their hosts (Figure 3a). This results in different levels of scramble competition inside the host egg or pupa, leading to a large variation in body size. Body-size variants of *N. vitripennis* were induced with adapted wasp-to-host ratios (Groothuis & Smid, 2017). The smallest offspring emerged after parasitism of 5 *C. vomitoria* pupae by 50 *N. vitripennis*, and the largest offspring after parasitism of 20 pupae by 10 females. In both cases, the

adult female wasps were given the opportunity to oviposit the pupae for 24 h and were thereafter removed.

Wasps of the genus *Trichogramma* assess host-egg size through antennal drumming of the host surface (Schmidt & Smith, 1985). To induce body-size variation in *T. evanescens*, we therefore partially masked the surface of some *M. sexta* eggs by placing them on 5-10 ml cooling 1% agarose (Sigma) in Petri dishes (Greiner Bio-One, 94x15 mm) as described before (Van der Woude & Smid, 2016). This partial masking of host eggs resulted in smaller host-egg surfaces available for size assessment by the wasps. Fewer eggs were laid inside these masked hosts, which developed into larger offspring than generally emerged from unmasked host eggs. To ensure that large and small wasps were both available, we used a combination of masked and unmasked eggs. We ensured that eggs were only parasitized once by one wasp.

For both species we created small and large size classes by visually inspecting body sizes. To determine body size of these classes, we sampled 100 large and 100 small female *T. evanescens* and *N. vitripennis* wasps from 2 separate generations. These wasps were CO_2-sedated and their body length was measured from head to abdomen tip, and from thorax to abdomen tip, using a microscope ocular with an internal reticle scale (Table 1). Wasps of both species had unlimited access to honey and water until use in the conditioning trials.

Conditioning procedures

Females of both wasp species were trained to remember an odour or colour as illustrated in Figure 3b. We used single classical conditioning trials as described before (Hoedjes et al., 2014b; Hoedjes et al., 2012). Wasps obtained a rewarding experience with a host (the unconditioned stimulus, US) while perceiving a conditioned stimulus (CS+); either a colour or odour. Next, the wasps received an unrewarding experience (absence of hosts) on a different conditioned stimulus (CS-); another colour or odour. Conditioning procedures were carried out reciprocally, using either two odours or two colours as conditioned stimuli. Half of the groups were conditioned using the first of the two conditioned stimuli as CS+ and the second as CS-, and the other half of the groups received the second of the two conditioned stimuli as CS+ and the first as CS-. In total, we conditioned 151 reciprocal groups of *N. vitripennis* and 198 reciprocal groups of *T. evanescens*.

Female *T. evanescens* (2 days old) were conditioned inside glass vials (7.5 cm long, 1.2 cm diameter) in groups of approximately 50 wasps. Pieces of filter paper (~1 cm^2) with a clutch of approximately 30 *M. brassicae* eggs were used as US. Female *N. vitripennis* (2 days old) were conditioned in Petri dishes (8.5 cm diameter) in groups of approximately 50 wasps. These dishes contained approximately 40 *C. vomitoria* pupae as US and were covered with filter paper.

The duration of the CS+ phase was 15 minutes for *T. evanescens* and 1 h for *N. vitripennis*. The difference in duration of the CS+ phase relates to the difference in time it takes the two species to start laying eggs in these particular host species. For *T. evanescens,* drilling in *M. brassicae* takes a short time and the wasps start oviposition within minutes after finding the host. They were removed after 15 minutes to ensure that they had sufficient time to start oviposition, but also remain motivated to find hosts during the subsequent memory retention tests. Initiating oviposition takes a longer time for *N. vitripennis*. During a one h-long experience on a *C. vomitoria* host, the wasps will drill into the pupa and start feeding from its contents (Hoedjes et al., 2014a).

After the CS+ phase, the wasps were removed from their hosts with an aspirator (with additional aid of fine tweezers for *T. evanescens*) and placed in clean vials or dishes on neutral backgrounds for a resting period of 15 minutes. This was followed by the CS- phase, which lasted for 15 minutes for both species.

Olfactory conditioning trials were performed using Royal Brand Bourbon Vanilla extract and Natural Chocolate extract as CS for *N. vitripennis*, and Royal Brand Bourbon Vanilla extract and Natural Coffee extract for *T. evanescens* (Nielsen-Massey Vanillas Intl., Leeuwarden, the Netherlands). These artificial odours were chosen because they represent neutral stimuli for the wasps, for which they do not show innate preferences. Chocolate and vanilla have previously been found to be most suitable for olfactory conditioning in *N. vitripennis* (Hoedjes et al., 2012). For *T. evanescens*, pilot experiments revealed that using coffee extract instead of chocolate induced higher memory retention levels (not shown). The extracts were placed on pieces of filter paper (~1 cm^2) in drops of 1 µl for *T. evanescens* and 5 µl for *N. vitripennis*, and placed inside the conditioning vial or dish during the CS+ and CS- phases.

Visual conditioning trials were performed with the colours blue and yellow as CS for both species (Clairefontaine Trophée 120 g/m^2 hues 1291 and 1292 for *T. evanescens* and hues 1247 and 1292 for *N. vitripennis*). Blue and yellow have previously been used as visual stimuli in conditioning experiments with *N. vitripennis* (Oliai & King, 2000). Using a slightly brighter shade of blue for *N. vitripennis* and a slightly darker shade for *T. evanescens* improved memory retention levels for both species during pilot experiments (not shown). For *T. evanescens*, the conditioning vials were placed in boxes (10.5x15.5 cm) that were lined with blue or yellow paper. For *N. vitripennis* the conditioning dishes were placed on blue or yellow paper. The conditioning procedures took place in areas that were shielded from environmental light, and lit by 4 fluorescent tubes (Philips Master TL5 H0 39W/865 for *T. evanescens* and Philips Master TL5 H0 39W/840 for *N. vitripennis*).

Memory retention tests

Memory retention (Figure 3c) was tested 1, 4 and 24 h after conditioning. Olfactory memory was retained longer than 24 h in *N. vitripennis*, and was therefore tested 1, 3 and 5 days after conditioning.

A third of each group of 50 wasps was tested at each time point, ensuring that each individual wasp was tested only once.

Olfactory memory of *N. vitripennis* was tested in the T-maze described by Hoedjes et al. (2012). An adapted version of this T-maze was used for *T. evanescens*, consisting of two transparent, polycarbonate tubes (1.6 cm diameter, 11 cm long) that connected smoothly to a 3 cm-long central aluminium tube. Both types of T-mazes contained a small opening to insert wasps and fine mesh to allow air flow to leave the T-maze. The distal ends were connected to Teflon tubes that contained a single glass capillary (ID 1.3 mm, Stuart SMP1/4, Bibby Scientific, Staffordshire, UK) for odour transmission on each side of the T-maze. One side contained a capillary filled with vanilla extract, and the other side contained chocolate extract in the T-maze for *N. vitripennis* and coffee extract in the T-maze for *T. evanescens*. Charcoal filtered, moisturized air (60–70% relative humidity) flowed past odour capillaries at 100 ml/min per side for *N. vitripennis* and 30 mL/min per side for *T. evanescens.*

Visual memory retention in *N. vitripennis* was tested in 40-cm-long polycarbonate tubes (3.6 cm diameter) (Kunststofshop, Zevenaar, the Netherlands). The lower half of the tubes was covered with blue paper on one side, and yellow paper on the other side. The central 5 cm were left transparent and contained a small hole for insertion of wasps.

Visual memory retention in *T. evanescens* was tested in a T-maze that was constructed from two glass vials (15 cm long, 1.8 cm diameter). The vials were connected by aluminium tubes similar to those that connected the olfactory T-maze. The setup was placed in a box (10.5 x 40 x 5 cm) lined with blue paper on one side and yellow paper on the other side.

All memory retention tests took place in areas that were shielded from environmental light. The olfactory T-maze for *N. vitripennis* was illuminated by a LED strip (Grandi ‘white’ 6000-6500K, 170 lm/m with 30 leds/m mounted against a white shelf 40 cm above the T-maze), the other T-mazes were lit by the same TL tubes that were used during conditioning. Wasps were inserted into the T-mazes with an aspirator. After 10 minutes, the number of wasps on each side of the T-maze was counted. Wasps in the central areas were considered as non-responding. The orientation of the T-mazes was reversed after two tests to prevent any bias from environmental influences.

Statistical analysis

Differences in body length between size classes were analysed with Welch two sample t-tests. Memory retention was expressed as performance index (PI) (Hoedjes et al., 2012). The PI was calculated by taking the fraction of wasps that made a choice for the odour or colour of their CS+, and subtracting the fraction of wasps from the other reciprocal group that made a choice for the odour or colour that they experienced as CS-. A PI for visual memory retention, for example, is calculated by subtracting the

fraction of wasps that received yellow as CS+ and chose blue, from the fraction of wasps that received blue as CS+ and chose blue.

The fractions that were used to calculate PIs were obtained as the estimate of the group means of the predicted response of generalized linear mixed models (GLMMs) with logit link function and binomial distribution. The models' dependent variables were the number of wasps on one side of the T-maze (on blue for visual memory retention tests, on coffee for olfactory memory retention tests with *T. evanescens*, and on chocolate for olfactory memory retention tests with *N. vitripennis*), with the total number of wasps making a choice as denominator. The model response of visual memory retention tests, for example, is therefore the fraction of wasps that chose blue over yellow. Including CS+ as fixed effect allowed to test for the effect of conditioning on the preference for the two odours or colours that were used as conditioned stimuli. Other fixed effects that were included in the model were time after conditioning, body size class and the interactions between fixed effects. Random effects were included to correct for conditioning date and the reciprocal conditioning pair the wasps belonged to.

To test if memory was formed, Bonferroni-corrected χ^2 pairwise comparisons were used to test the effect of CS+ on the preference for conditioned stimuli. In case of a significant main effect of body size on memory retention, post-hoc χ^2 pairwise comparisons tested if memory retention differed between the size classes within the different time points after conditioning. Response rates of wasps were determined by defining another GLMM using the fraction of wasps making a choice out of the total number of wasps inserted as dependent variable. Fixed factors were size and time after conditioning. Differences in response rate of small and large wasps were determined using Bonferroni-corrected χ^2 pairwise comparisons. Statistical analyses were performed in R 3.0.2 with packages lme4 (Bates et al., 2014), phia (De Rosario-Martinez) and lsmeans (Lenth, 2014).

Ethical note

In addition to the general rearing and handling procedures given under "insects", we here provide additional information concerning animal welfare. This research consisted of non-invasive observations of natural behaviour and can be considered to be minimally disturbing to the studied insects. For studies on insects, no ethical approval is required in The Netherlands. The wasp species parasitized the eggs or pupa, but not larvae or adults, thereby avoiding potential distress of parasitized hosts. Hosts eggs and pupae were kept at 4°C until use, in plastic bottles (eggs) or plastic containers with fresh saw dust (pupae). Host species were either supplied at 4°C, by commercial suppliers (*Ephestia kuehniella* eggs and *Calliphora vomitoria* larvae), from the Max Planck Institute for Chemical Ecology, Jena, Germany (pupa of *Manduca sexta*) or reared in our laboratory in compartments with dimensions of 40x45x65cm per approximately 200 larvae and 60x20 cm cylinders per approximately 50 adults with unlimited access to food and water (*Mamestra brassicae*). Up to 10 adult *Manduca sexta* moths were allowed to mate

and to oviposit in a cage of dimensions 50x50x50cm. *Calliphora vomitoria* larvae were allowed to pupate directly after purchase at room temperature in a plastic container of dimensions 20x22x17cm in sawdust per 750ml of larvae, covered by cotton fabric. Prior to the experiments and in-between the conditioning and memory retention tests, wasps were kept in glass tubes with dimensions of 150x16 mm, with approximately up to 500 wasps (*T. evanescens*) or plastic tubes of dimensions 94x27mm and approximately up to 350 wasps (*N. vitripennis*) at standard laboratory conditions as described under 'insects' with access to honey and water. They were removed from their rearing vials by gentle aspiration directly before use in experiments. For body length measurements, wasps were anaesthetized with the lowest concentrations of CO_2 that resulted in full anaesthesia, and carefully placed in clean vials to recover before being returned to their rearing vials. Conditioning procedures required the wasps to be removed before completing oviposition. This removal was done by aspiration, but when the ovipositor of *T. evanescens* was placed deep inside the host, these wasps had to be held by their wings with tweezers before gently being pulled in a vertical direction. Care was taken to remove all wasp from ovipositing positions without harming the ovipositor or wings, or any other body part. The wasps did not show signs of distress after being removed from their hosts or during or after other elements of the experiments. After completion of the memory retention tests, the wasps were carefully collected by gentle aspiration. In total 7550 female *N. vitripennis*, 9900 female *T. evanescens*, 6040 *C. vomitoria* pupae and 5990 *M. brassicae* eggs were used for conditioning experiments and afterwards euthanized in a −20 °C freezer overnight.

Results

Body size variation

Body length ranged between 0.367–0.967 mm in *T. evanescens* and 1.375–2.825 mm in *N. vitripennis* (Table 1, Figure 2b). When body length was measured from the thorax to abdomen tip (thereby excluding the head), this length ranged between 0.311–0.856 mm in *T. evanescens* and 1.175–2.475 mm in *N. vitripennis.* Average body length (±SD) in *T. evanescens* was larger (0.745 ± 0.054 mm) in the large size class than in the small size class (0.521 ± 0.064 mm; $t(192.51)=26.766$, $P<0.001$). In *N. vitripennis,* average body length was 2.634 ± 0.085 mm in the large size class and 1.681 ± 0.099 mm in the small size class, and significantly different between the size classes ($t(193.44)=72.929$, $P<0.001$). Average thorax-abdomen length in *T. evanescens* was 0.654 ± 0.052 mm in the large size class, and 0.444 ± 0.058 mm in the small size class, and also differed between the size classes ($t(192.26)=26.880$, $P<0.001$). In *N. vitripennis,* average thorax-abdomen length was 2.330 ± 0.078 mm in the large size class and 1.450 ± 0.094 mm in the small size class, also significantly different ($t(191.71)=71.889$, $P<0.001$).

Olfactory memory retention in N. vitripennis

In total, 2025 *N. vitripennis* responded in the olfactory memory retention tests (79 reciprocal groups). A single olfactory conditioning trial resulted in memory retention in *N. vitripennis* (χ^2_1=150.075, p<0.001; Figure 4a), which did not decrease over time after conditioning (χ^2_2=4.789, p=0.091). Small wasps had a lower level of memory retention than large wasps (χ^2_1=15.473, *P*<0.001). There were no differences in duration of memory retention between wasps of different sizes (χ^2_1=0.981, *P*=0.612).

Small and large *N. vitripennis* retained olfactory memory up to 5 days after conditioning. One day after conditioning, small wasps showed a PI (±SE) of 23.90 ± 6.44% (χ^2_1 = 19.536, *P*< 0.001) and large wasps of 46.39 ± 6.00% (χ^2_1 = 60.356, *P*< 0.001). Three days after conditioning, small wasps showed a PI of 22.88 ± 6.54% (χ^2_1 = 18.201, *P* < 0.001) and large wasps of 36.41 ± 6.57% (χ^2_1 = 36.694, *P*< 0.001). Five days after conditioning, small wasps showed a PI of 16.25 ± 6.50% (χ^2_1 = 10.349, *P*= 0.008) and large wasps of 31.79 ± 6.75% (χ^2_1 = 28.189, *P*< 0.001). The PI was significantly lower for small wasps than for large wasps one day after conditioning (χ^2_1 = 8.998, *P* = 0.003) and five days after conditioning (χ^2_1 = 4.258, *P*= 0.040), but did not differ between small and large wasps three days after conditioning (χ^2_1 = 3.192, *P*= 0.074).

Visual memory retention in N. vitripennis

In total, 1964 *N. vitripennis* responded in the visual memory retention tests (72 reciprocal groups). A single visual conditioning trial resulted in memory retention in *N. vitripennis* (χ^2_1=105.495, *P*<0.001; Figure 4b), which decreased over time after conditioning (χ^2_2=31.116, *P*<0.001). Small wasps had a lower level of memory retention than large wasps (χ^2_1=7.731, *P*=0.005). There were no differences in the duration of memory retention between wasps of different sizes (χ^2_1=0.831, *P*=0.660).

Small and large *N. vitripennis* retained visual memory up to 4 h after conditioning. One h after conditioning, small wasps showed a PI (±SE) of 24.97 ± 6.16% (χ^2_1 = 20.724, *P*< 0.001) and large wasps of 40.51 ± 5.67% (χ^2_1 = 53.577, *P* < 0.001). Four h after conditioning, small wasps showed a PI of 27.70 ± 6.51% (χ^2_1 = 23.621, *P*< 0.001) and large wasps of 37.16 ± 6.62% (χ^2_1 = 43.449, *P*< 0.001). Twenty-four h after conditioning, small wasps showed a PI of 2.94 ± 6.39% (χ^2_1 = 0.296, *P*= 1.000) and large wasps of 11.82 ± 5.94% (χ^2_1 = 4.402, *P*= 0.215). The PI was significantly lower for small wasps than for large wasps one h after conditioning (χ^2_1 = 5.122, *P*= 0.024), but did not differ between small and large wasps four h after conditioning (χ^2_1 = 2.132, *P*= 0.144) and twenty-four h after conditioning (χ^2_1 = 1.309, *P*= 0.253).

Olfactory memory retention in T. evanescens

In total, 2733 *T. evanescens* responded in the olfactory memory retention tests (107 reciprocal groups). A single olfactory conditioning trial resulted in memory retention in *T. evanescens* (χ^2_1=52.213, *P*<0.001; Figure 4c), which decreased over time after conditioning (χ^2_2=7.381, *P*=0.025). Small and large wasps form the same level of memory retention (χ^2_1=0.922, *P*=0.337). There were no differences in the duration of memory retention between wasps of different sizes (χ^2_1=0.509, *P*=0.775).

Small *T. evanescens* retained olfactory memory up to 4 h after conditioning, while 4-h memory was no longer significantly different from 0 in large wasps. One h after conditioning, small wasps showed a PI (±SE) of 20.83 ± 5.10% (χ^2_1=20.195, *P*<0.001) and large wasps of 19.64 ± 5.42% (χ^2_1=16.140, *P*<0.001). Four h after conditioning, small wasps showed a PI of 18.44 ± 5.41% (χ^2_1=14.422, *P*<0.001) and large wasps of 11.16 ± 5.14% (χ^2_1=6.199, *P*=0.077). Twenty-four h after conditioning, small wasps showed a PI of 8.68 ± 5.57% (χ^2_1=2.996, *P*=0.501) and large wasps of 5.53 ± 5.28% (χ^2_1=1.385, *P*=1.000).

Visual memory retention in T. evanescens

In total, 3002 *T. evanescens* responded in the visual memory retention tests (91 reciprocal groups). A single visual conditioning trial resulted in memory retention in *T. evanescens* (χ^2_1=94.529, *P*<0.001; Figure 4d), which decreased over time after conditioning (χ^2_2=23.717, *P*<0.001). Small and large wasps form the same level of memory retention (χ^2_1=0.006, *P*=0.937). There were no differences in the duration of memory retention between wasps of different sizes (χ^2_1=0.509, *P*=0.776).

Small and large *T. evanescens* retained visual memory up to 4 h after conditioning. One h after conditioning, small wasps showed a PI (±SE) of 29.05 ± 6.60% (χ^2_1=29.301, *P*<0.001) and large wasps of 31.48 ± 6.16% (χ^2_1=46.351, *P*<0.001). Four h after conditioning, small wasps showed a PI of 18.22 ± 6.66% (χ^2_1=13.845, p=0.001) and large wasps of 20.51 ± 6.41% (χ^2_1=20.761, *P*<0.001). Twenty-four h after conditioning, small wasps showed a PI of 10.71 ± 6.54% (χ^2_1=5.322, *P*=0.126) and large wasps of 7.20 ± 6.39% (χ^2_1=3.281, *P*=0.420).

Response rate

Response rate was defined as the percentage of wasps making a choice, out of the total number of wasps that were introduced into the T-maze. Response rate was lower in small than in large *T. evanescens* (visual: χ^2_1=15.840, p<0.001; olfactory: χ^2_1=25.800, *P*<0.001). Small *T. evanescens* showed a response rate (±SE) of 78.63 ± 2.51% during visual memory retention tests and 76.64 ± 1.85% during olfactory memory retention tests. Large *T. evanescens* showed a response rate of 84.42 ± 1.98% during visual memory retention tests and 84.30 ± 1.45% during olfactory memory retention tests. In *N. vitripennis*, small wasps showed a lower response rate than large wasps during visual retention tests (χ^2_1=4.339, *P*=0.037), and a higher response rate than large wasps during olfactory memory retention tests

(χ^2_1=18.315, P<0.001). Small *N. vitripennis* showed a response rate (±SE) of 91.67 ± 0.95% during visual memory retention tests and 85.49 ± 1.12% during olfactory memory retention tests. Large *N. vitripennis* showed a response rate of 94.20 ± 0.81% during visual memory retention tests and 78.49 ± 1.41% during olfactory memory retention tests.

Discussion

We expected from the observed isometry in *T. evanescens* and a combination of isometry and allometry in *N. vitripennis*, that small individuals would show compromised memory performance compared to large individuals. We found that such a cognitive cost of small brains was apparent in *N. vitripennis*, but that it was absent in the smaller wasp species *T. evanescens*. For both species, we used inbred iso-female strains to exclude inter-individual genetic effects. The results of the present study therefore suggest that developmental plasticity in brain and body size differentially affects brain performance in *N. vitripennis* and *T. evanescens*.

In *N. vitripennis*, the level of visual and olfactory memory retention was significantly lower in small wasps than in large conspecifics. This could not be explained by a difference in host-searching activity; small *N. vitripennis* showed a lower response rate than large wasps during visual memory tests, but a higher response rate than large wasps during the olfactory memory tests. The duration of memory retention did not differ between small and large *N. vitripennis*. The cognitive costs of brain scaling in *N. vitripennis* may therefore mainly be reflected in the level of memory retention, rather than in the type of memory or its retention over time.

The present study shows that body size does not affect memory performance in *T. evanescens*, despite the isometric brain scaling that occurs in this species (Van der Woude et al., 2013). Hence, that small *T. evanescens* showed similar levels and duration of memory retention as large conspecifics is surprising. These results may suggest that for this species, the costs of the extreme developmental size plasticity of the brains are not reflected in this aspect of cognitive performance. The different effect of body size on memory performance between *T. evanescens* and *N. vitripennis* could relate to ecological differences between the two species, and to differences in developmental plasticity in neural architecture, on which we elaborate below.

Ecological importance of learning

The procedures used for conditioning and measuring the PI are simplified versions of the oviposition learning and host foraging tasks that the wasps encounter in natural circumstances, which should be kept in mind when considering implications for the ecological importance of learning. Nevertheless, the results of the present study show that *N. vitripennis* and *T. evanescens* are capable of forming both visual and olfactory memory, which can be of ecological importance for these wasps. Both *N. vitripennis* and *T. evanescens* continue to produce and mature eggs throughout their life, and will therefore need to

continue searching for suitable hosts (Jacob and Boivin, 2005; Rivero and West, 2002). The two species are also both gregarious generalists that exploit a large variety of host species (Huigens et al., 2009; Hoedjes et al., 2012). Learning can allow them to focus their searching activities on the particular host species that are present in their current environment (Hoedjes et al., 2011). Our study revealed that *N. vitripennis* retained olfactory memory longer than visual memory, which suggests that olfactory cues play a larger role during host searching than visual ones. In contrast, the similarity in memory retention of visual and olfactory cues in *T. evanescens* could suggest that these wasps use both visual and olfactory cues to find suitable hosts.

Trichogramma evanescens wasps differ from *N. vitripennis* in the strategy that they apply to find their hosts. Female *T. evanescens* have been shown to mount mated female butterflies and use them as means of transportation to the butterflies' egg-laying sites (Huigens et al., 2009). This phoresy behaviour enables wasps of the genus *Trichogramma* to find and parasitize freshly-laid host eggs, despite the limited control these tiny wasps have over the direction of their flight (Fatouros et al., 2005). Phoresy may reduce the amount of energy and neural capacity that needs to be allocated to navigation and flight, and allow increased investment in the cognitive and sensory abilities that are required to locate lepidopteran host species. This could underlie the similarities in memory performance of small and large *T. evanescens.* In contrast, it may be more adaptive for small *N. vitripennis* to economize on memory performance, and maintain energy, motor capacities and navigational functions to actively search for hosts.

Memory performance could have been affected by the ecology of the host species that we used as unconditioned stimuli. There are various characteristics that determine how rewarding a particular host is, such as clutch size, host size, nutritional quality, and whether the host has already been parasitized (Kruidhof et al., 2012). For *T. evanescens,* the reward value of the host determines how long memory is retained (Kruidhof et al., 2012). Long-term memory is formed after an oviposition experience on a clutch of *Pieris brassicae* eggs, but memory is retained shorter after an oviposition experience on *Pieris rapae* eggs, which are somewhat smaller and deposited as single eggs on multiple plant species (Kruidhof et al., 2012). The reward value of the host does not affect memory performance of *N. vitripennis* (Hoedjes et al., 2014a). Oviposition into three differently-sized host species results in the emergence of different numbers and sizes of offspring, but using these differently-sized hosts as unconditioned stimuli does not result in differences in memory retention. Hence, *T. evanescens* and *N. vitripennis* appear to have evolved different strategies of dealing with ecological variation in quality or suitability of their host species. Oviposition learning may be less dependent on ecological conditions for *N. vitripennis* than for *T. evanescens*. It is interesting that the opposite is the case for body-size variation.

Plasticity in brain morphology

The lower memory retention levels in small *N. vitripennis* could indicate that small and large adults differentially invest in specific brain areas. Groothuis and Smid (2017) compared relative neuropil

volumes for *N. vitripennis* females that were similar in size range and obtained in the same way as individuals in the present study. Indeed, they found that the mushroom bodies and optic lobes are relatively smaller in small than in large wasps, whereas relative volume of other neuropils remains the same or becomes relatively larger. The mushroom bodies are the location where different types of sensory pathways converge that convey the US and CS, and there is overwhelming evidence that they are essential for learning and memory formation (Perry & Barron, 2012). The finding that scramble competition induces developmental programmes that lead to smaller wasps with smaller relative mushroom body volumes (Groothuis & Smid, 2017) supports our results of small individuals having lower memory performance. Similar data for mushroom-body volume in *T. evanescens* are currently not available, but the results of the present study could indicate that relative mushroom-body volume is maintained in small and large *T. evanescens*.

First explorations of the neural architecture of *T. evanescens* revealed some striking similarities in numbers of aminergic neurons, as well as in the number of olfactory glomeruli in the antennal lobe of small and large individuals, whereas the size of these neural components does relate to body size. In the antennal lobe, olfactory glomeruli were found to be relatively larger in wasps with larger brain volumes, but differently-sized wasps had the same number of glomeruli in their antennal lobes (Van der Woude and Smid, 2016). Similarly, small and large *T. evanescens* differed in the diameter of neuronal cell bodies that express serotonin, dopamine and octopamine, but did not differ in the number of these neurons (Van der Woude and Smid, 2017). These first explorations suggest that the complexity of the brains of small and large *T. evanescens* may be similar, which supports the similarities in memory retention levels of these wasps. In *N. vitripennis*, the number and size of octopaminergic neurons has been studied but only in large individuals of this species (Haverkamp and Smid, 2014). Future studies should reveal if *N. vitripennis* evolved a different strategy than *T. evanescens*, which could involve reduced numbers of neurons and olfactory glomeruli in smaller individuals.

Another aspect that may affect the different cognitive consequences of brain-body-size scaling are the relative frequencies of the different body sizes that occur in nature. Both are gregarious species, which can adapt the number of eggs they lay into a certain host to obtain the most optimal balance in number, sex and size of their offspring (Salt, 1940; Charnov and Skinner, 1984; Ivens et al., 2009). *Trichogramma evanescens* females lay multiple eggs even in small host eggs (van der Woude and Smid, 2017; Waage and Ming (1984), suggesting that higher numbers of smaller phenotypes result in higher fitness. In *N. vitripennis*, the number of eggs laid per host rather yields the larger phenotypes of the range that we obtained in this study (Hoedjes et al., 2014a). The smallest host eggs, and hence the smaller wasps may be much more abundant than the larger hosts and the larger wasps for *T. evanescens*, which may attenuate the evolutionary pressure to develop specific mechanisms that optimize brain performance for larger wasps. The opposite may be the case for *N. vitripennis*. However, under such a scenario, we would not expect to observe isometric brain body size scaling, since this actually reflects

specific adaptations that require a higher level of plasticity in brain development than negative allometry. Isometric brain-body size scaling implies that large individuals have brains that are much larger than expected in a situation with allometric scaling. The fact that such mechanisms have evolved does not suggest that the absence of improved cognition is a result of limited evolutionary pressure due to the lower frequencies of such larger phenotypes. There must selective advantages for having these large brains that outweigh the high energetic costs. The results of the present study indicate that these benefits may not be cognitive in *T. evanescens*: large brains do not provide higher memory retention levels. Instead, the trade-offs of isometric brain scaling must be sought in other aspects of brain performance or fitness. These could relate, for example, to the smaller size of neuronal cell bodies in the smallest *T. evanescens* (Van der Woude and Smid, 2017). The limited volume of these cell bodies may restrict the number of energy-generating mitochondria and could enforce chromatin to be tightly packed, which may obstruct transcription and neural processing. These modifications may affect the longevity of the smallest *T. evanescens*, and larger conspecifics could avoid these costs by investing more in neuronal cell body size. Indeed, larger *T. evanescens* have a higher longevity and fecundity than smaller individuals (Waage and Ming, 1984; Doyon and Boivin, 2005).

Conclusion

The results of our study indicate that different evolutionary pressures shaped the cognitive consequences of extreme brain-scaling strategies. The smallest *T. evanescens* maintain memory performance under isometric brain scaling, which may be facilitated by a developmental strategy that reduces the size of neural components, while neural complexity is maintained. A possible trade-off of brain isometry in *T. evanescens* must be sought in brain properties different from memory retention (this paper) and olfaction (Van der Woude & Smid, 2016), and could relate to neuronal cell body size. The larger parasitic wasp species *N. vitripennis* is unable to maintain memory retention levels at small body sizes, which may relate to previous findings of relatively smaller mushroom bodies in small *N. vitripennis* (Groothuis & Smid, 2017). It may be more adaptive for small *N. vitripennis* to maintain investment in other aspects of brain performance, at the cost of memory performance. Future studies will need to reveal if the similarities in memory retention level in small and large *T. evanescens* can be explained by maintained relative mushroom body size, and if isometric brain scaling causes costs and benefits in other traits. A comparison of neural complexity in small and large *N. vitripennis* and *T. evanescens* may reveal which mechanisms enable their brain-scaling strategies, and explain the cognitive consequences for the smallest insects.

Acknowledgements

We thank Jessica de Bruijn, Lucía Martín de Bernardo Gisbert, Clara Galves-Orjol and Alexandra Roux for help with designing and conducting experiments; Marcel Dicke and Jitte Groothuis and three

anonymous reviewers for constructive comments on a previous version of this article; Jitte Groothuis for providing the photographs in Figure 1, Maartje Liefting (Free University of Amsterdam) for sharing the visual conditioning and memory retention procedure for *N. vitripennis*; Marjolein Kruidhof (Wageningen Plant Research) for statistical advice; the Max Planck Institute for Chemical Ecology (Jena, Germany) for providing *M. sexta* pupae; Léon Westerd, Frans van Aggelen and André Gidding for culturing *M. brassicae*; and the experimental farm of Wageningen University (Unifarm) for growing the tobacco plants. This work was supported by NWO PE&RC Graduate Program grant 022.002.004 (to EW) and by NWO Open Competition grant 820.01.012 (to HS).

References

Aiello, L. C., & Wheeler, P. (1995). The expensive-tissue hypothesis - the brain and the digestive system in human and primate evolution. *Current Anthropology, 36*, 199-221. doi:10.1086/204350

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2014). lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7. Retrieved from http://CRAN.R-project.org/package=lme4

Breeuwer, J. A. J., and J. H. Werren, 1995. Hybrid breakdown between two haplodiploid species: the role of nuclear and cytoplasmic genes. Evolution 49: 705–717.

Charnov, E. L., & Skinner, S. W. (1984). Evolution of Host Selection and Clutch Size in Parasitoid Wasps. *The Florida Entomologist, 67*, 5–21.

Chittka, L., & Niven, J. (2009). Are bigger brains better? *Current Biology, 19*(21), R995-R1008. doi:10.1016/j.cub.2009.08.023

De Rosario-Martinez, H. phia: Post-Hoc Interaction Analysis. R package version 0.1-5. Retrieved from http://CRAN.R-project.org/package=phia

Doyon J, Boivin G. (2005). The effect of development time on the fitness of female *Trichogramma evanescens*. *Journal of Insect Science, 5:4*.

Eberhard, W. G., & Wcislo, W. T. (2011). Grade changes in brain–body allometry: morphological and behavioural correlates of brain size in miniature spiders, insects and other invertebrates. In J. Casas (Ed.), *Advances in Insect Physiology, Vol. 60, pp. 155-214* Burlington: Academic Press.

Fatouros NE, Huigens ME, van Loon JJA, Dicke M, Hilker M. (2005). Chemical communication: butterfly anti-aphrodisiac lures parasitic wasps. *Nature 433*, 704.

Feener, D. H., Lighton, J. R. B., & Bartholomew, G. A. (1988). Curvilinear allometry, energetics and foraging ecology: a comparison of leaf-cutting ants and army ants. *Functional Ecology, 2*, 509-520. doi:10.2307/2389394

Gonda, A., Herczeg, G., & Merila, J. (2011). Population variation in brain size of nine-spined sticklebacks (*Pungitius pungitius*) - local adaptation or environmentally induced variation? *BMC Evolutionary Biology, 11*, 75. doi:10.1186/1471-2148-11-75

552 Groothuis, J., & Smid, H. M. (2017). *Nasonia* parasitic wasps escape from Haller's rule by diphasic,
553 partially isometric brain-body size scaling and selective neuropil adaptations. *Brain Behavior*
554 *and Evolution, 90(3),* 243-254: doi:10.1159/000480421
555 Harvey, P. H., & Krebs, J. R. (1990). Comparing brains. *Science, 249,* 140-146.
556 doi:10.1126/science.2196673
557 Haverkamp A, Smid HM. (2014). Octopamine-like immunoreactive neurons in the brain and
558 subesophageal ganglion of the parasitic wasps *Nasonia vitripennis* and *N. giraulti*. *Cell Tissue*
559 *Research 358,* 313-329.
560 Hoedjes, K. M., Kralemann, L. E. M., van Vugt, J. J. F. A., Vet, L. E. M., & Smid, H. M. (2014a).
561 Unravelling reward value: the effect of host value on memory retention in *Nasonia* parasitic
562 wasps. *Animal Behaviour, 96,* 1-7. doi:10.1016/j.anbehav.2014.07.013
563 Hoedjes, K. M., Kruidhof, H. M., Huigens, M. E., Dicke, M., Vet, L. E., & Smid, H. M. (2011). Natural
564 variation in learning rate and memory dynamics in parasitoid wasps: opportunities for
565 converging ecology and neuroscience. *Proceedings: Biological Sciences, 278,* 889-897.
566 doi:10.1098/rspb.2010.2199
567 Hoedjes, K. M., & Smid, H. M. (2014). Natural variation in long-term memory formation among
568 *Nasonia* parasitic wasp species. *Behavioural Processes, 105,* 40-45.
569 doi:10.1016/j.beproc.2014.02.014
570 Hoedjes, K. M., Smid, H. M., Vet, L. E. M., & Werren, J. H. (2014b). Introgression study reveals two
571 quantitative trait loci involved in interspecific variation in memory retention among *Nasonia*
572 wasp species. *Heredity, 113,* 542-550. doi:10.1038/hdy.2014.66
573 Hoedjes, K. M., Steidle, J. L., Werren, J. H., Vet, L. E., & Smid, H. M. (2012). High-throughput
574 olfactory conditioning and memory retention test show variation in *Nasonia* parasitic wasps.
575 *Genes Brain and Behavior, 11,* 879-887. doi:10.1111/j.1601-183X.2012.00823.x
576 Huigens, M. E., Pashalidou, F. G., Qian, M. H., Bukovinszky, T., Smid, H. M., van Loon, J. J. A., Dicke,
577 M., Fatouros, N. E. (2009). Hitch-hiking parasitic wasp learns to exploit butterfly
578 antiaphrodisiac. *Proceedings of the National Academy of Sciences of the United States of*
579 *America, 106,* 820-825. doi:10.1073/pnas.0812277106
580 Ivens, A. B. F., Shuker, D. M., Beukeboom, L. W., & Pen, I. (2009). Host acceptance and sex allocation
581 of Nasonia wasps in response to conspecifics and heterospecifics. *Proceedings: Biological*
582 *Sciences / The Royal Society, 276,* 3663–3669.
583 Isler, K., Christopher Kirk, E., Miller, J. M., Albrecht, G. A., Gelvin, B. R., & Martin, R. D. (2008).
584 Endocranial volumes of primate species: scaling analyses using a comprehensive and reliable
585 data set. *Journal of Human Evolution, 55,* 967-978. doi:10.1016/j.jhevol.2008.08.004
586 Jacob S, Boivin G. (2005). Costs and benefits of polyandry in the egg parasitoid *Trichogramma*
587 *evanescens* Westwood (Hymenoptera: Trichogrammatidae). *Biol Control 32,* 311-318.

588 Keasar, T., Ney-Nifle, M., & Mangel, M. (2000). Evidence for learning of visual host-associated cues
589 in the parasitoid wasp *Trichogramma thalense*. *Israel Journal of Zoology, 46*, 243-247.
590 doi:10.1092/K08h-R8bg-Vt2u-F1hn
591 Kotrschal, A., Rogell, B., Bundsen, A., Svensson, B., Zajitschek, S., Brannstrom, I., Immler, S.,
592 Maklakov, A. A., Kolm, N. (2013). Artificial selection on relative brain size in the guppy
593 reveals costs and benefits of evolving a larger brain. *Current Biology, 23*, 168-171.
594 doi:10.1016/j.cub.2012.11.058
595 Kruidhof, H. M., Pashalidou, F. G., Fatouros, N. E., Figueroa, I. A., Vet, L. E., Smid, H. M., & Huigens,
596 M. E. (2012). Reward value determines memory consolidation in parasitic wasps. *PloS One,*
597 *7*, e39615. doi:10.1371/journal.pone.0039615
598 Kruska, D. (1996). The effect of domestication on brain size and composition in the mink (*Mustela*
599 *vison*). *Journal of Zoology, 239*, 645-661. doi:10.1111/j.1469-7998.1996.tb05468.x
600 Kühsel S, Brückner A, Schmelzle S, Heethoff M, Blüthgen N (2017) Surface area-volume ratios
601 in insects. *Insect Sci* 24, 829–841.
602 Lenth, R. V. (2014). lsmeans: Least-Squares Means. R package version 2.11 Retrieved from
603 http://CRAN.R-project.org/package=lsmeans
604 Margulies, C., Tully, T., & Dubnau, J. (2005). Deconstructing memory in *Drosophila*. *Current Biology,*
605 *15*, R700-R713. doi:10.1016/j.cub.2005.08.024
606 Mery, F., & Kawecki, T. J. (2005). A cost of long-term memory in *Drosophila*. *Science, 308*, 1148.
607 doi:10.1126/science.1111331
608 Oliai, S. E., & King, B. H. (2000). Associative learning in response to color in the parasitoid wasp
609 *Nasonia vitripennis* (Hymenoptera: Pteromalidae). *Journal of Insect Behavior, 13*, 55-69.
610 doi:10.1023/A:1007763525685
611 Pagel, M. D., & Harvey, P. H. (1989). Taxonomic differences in the scaling of brain on body weight
612 among mammals. *Science, 244*, 1589-1593. doi:10.1126/science.2740904
613 Perry, C. J., & Barron, A. B. (2012). Neural mechanisms of reward in insects. *Annual Review of*
614 *Entomology, 58,* 543-562 doi:10.1146/annurev-ento-120811-153631
615 Peters, R. S. (2010). Host range and offspring quantities in natural populations of *Nasonia vitripennis*
616 (Walker, 1836) (Hymenoptera: Chalcidoidea: Pteromalidae). *Journal of Hymenoptera*
617 *Research, 19,* 128-138.
618 Rensch, B. (1948). Histological changes correlated with evolutionary changes of body size. *Evolution,*
619 *2*, 218-230. doi:10.2307/2405381
620 Rivero A, West SA. (2002). The physiological costs of being small in a parasitic wasp. *Evolutionary*
621 *Ecology Research 4,* 407-420.
622 Riveros, A. J., & Gronenberg, W. (2010). Sensory allometry, foraging task specialization and resource
623 exploitation in honeybees. *Behavioral Ecology and Sociobiology, 64*, 955-966.
624 doi:10.1007/s00265-010-0911-6

Roth, G., & Dicke, U. (2005). Evolution of the brain and intelligence. *Trends in Cognitive Sciences, 9*, 250-257. doi:10.1016/j.tics.2005.03.005

Salt, G. (1940). Experimental studies in insect parasitism. VII. The effects of different hosts on the parasite *Trichogramma evanescens* West. (Hym. Chalcidoidea). *Proceedings of the Royal Entomological Society of London. Series A, General Entomology, 15*, 81-95. doi:10.1111/j.1365-3032.1940.tb00575.x

Schmidt, J. M., & Smith, J. J. B. (1985). Host volume measurement by the parasitoid wasp *Trichogramma minutum*: The roles of curvature and surface area. *Entomologia Experimentalis et Applicata, 39*, 213-221. doi:10.1111/j.1570-7458.1985.tb00462.x

Schurmann, D., Sommer, C., Schinko, A. P. B., Greschista, M., Smid, H., & Steidle, J. L. M. (2012). Demonstration of long-term memory in the parasitic wasp *Nasonia vitripennis*. *Entomologia Experimentalis et Applicata, 143*, 199-206. doi:10.1111/j.1570-7458.2012.01253.x

Seid, M. A., Castillo, A., & Wcislo, W. T. (2011). The allometry of brain miniaturization in ants. *Brain Behavior and Evolution, 77*, 5-13. doi:10.1159/000322530

Snell-Rood, E. C., Papaj, D. R., & Gronenberg, W. (2009). Brain size: a global or induced cost of learning? *Brain Behavior and Evolution, 73*, 111-128. doi:10.1159/000213647

Striedter, G. F. (2005). *Principles of brain evolution*. Sunderland, Massachusetts USA: Sinauer Associates, Inc.

Stuermer, I. W., Plotz, K., Leybold, A., Zinke, O., Kalberlah, O., Samjaa, R., & Scheich, H. (2003). Intraspecific allometric comparison of laboratory gerbils with Mongolian gerbils trapped in the wild indicates domestication in *Meriones unguiculatus* (Milne-Edwards, 1867) (Rodentia : Gerbillinae). *Zoologischer Anzeiger, 242*, 249-266. doi:10.1078/0044-5231-00102

Van der Woude, E., & Smid, H. M. (2016). How to escape from Haller's rule: olfactory system complexity in small and large *Trichogramma evanescens* parasitic wasps. *Journal of Comparative Neurology, 524*, 1876-1891. doi:10.1002/cne.23927

Van der Woude E, Smid HM. (2017). Effects of isometric brain-body size scaling on the complexity of monoaminergic neuron networks in a minute parasitic wasp. *Brain Behavior and Evolution 89*, 185-194 doi: 10.1159/000468974

Van der Woude, E., Smid, H. M., Chittka, L., & Huigens, M. E. (2013). Breaking Haller's rule: brain-body size isometry in a minute parasitic wasp. *Brain Behavior and Evolution, 81*, 86-92. doi:10.1159/000345945

Waage JK, Ming N. 1984. The reproductive strategy of a parasitic wasp: I. Optimal progeny and sex allocation in *Trichogramma evanescens*. *Journal of Animal Ecology 53*, 401-415.

Wehner, R., Fukushi, T., & Isler, K. (2007). On being small: brain allometry in ants. *Brain Behavior and Evolution, 69*, 220-228. doi:10.1159/000097057

Werren, J. H., & Loehlin, D. W. (2009). The parasitoid wasp *Nasonia*: an emerging model system with haploid male genetics. *Cold Spring Harbor protocols*, *2009*(10), pdb.emo134-pdb.emo134. doi:10.1101/pdb.emo134

Willemet R (2013) Reconsidering the evolution of brain, cognition, and behavior in birds and mammals. *Frontiers in Psychology 4*, 396.

Body length values of small and large wasps

	T. evanescens		*N. vitripennis*	
	Large	Small	Large	Small
Average body length (mm)	0.745 ± 0.054	0.521 ± 0.064	2.634 ± 0.085	1.681 ± 0.099
Average thorax-abdomen length (mm)	0.654 ± 0.052	0.444 ± 0.058	2.330 ± 0.078	1.450 ± 0.094
Body length range (mm)	0.644 ± 0.967	0.367 - 0.633	2.400 - 2.825	1.375 - 1.900
Thorax-abdomen length range (mm)	0.556 ± 0.856	0.311 - 0.556	2.150 - 2.475	1.175 - 1.650

Table 1 Body length values of large and small body size classes of *T. evanescens* and *N. vitripennis* (n=100 in each group). Shown are mean ± SD and total range of body lengths measured from head to abdomen tip, and from thorax to abdomen tip.

Captions to figures

Figure 1. Phenotypic plasticity in body size, showing large (open arrows) and small (black arrows) wasps of the species used in this study. **(a)** *N. vitripennis* on a *C. vomitoria* host pupa. **(b)** *T. evanescens* on an *M. sexta* host egg **(c).** A small *T. evanescens* on the head of a large *N. vitripennis,* illustrating the difference in body size between the two species. Scale bars indicate 0.5 mm. Pictures: Jitte Groothuis.

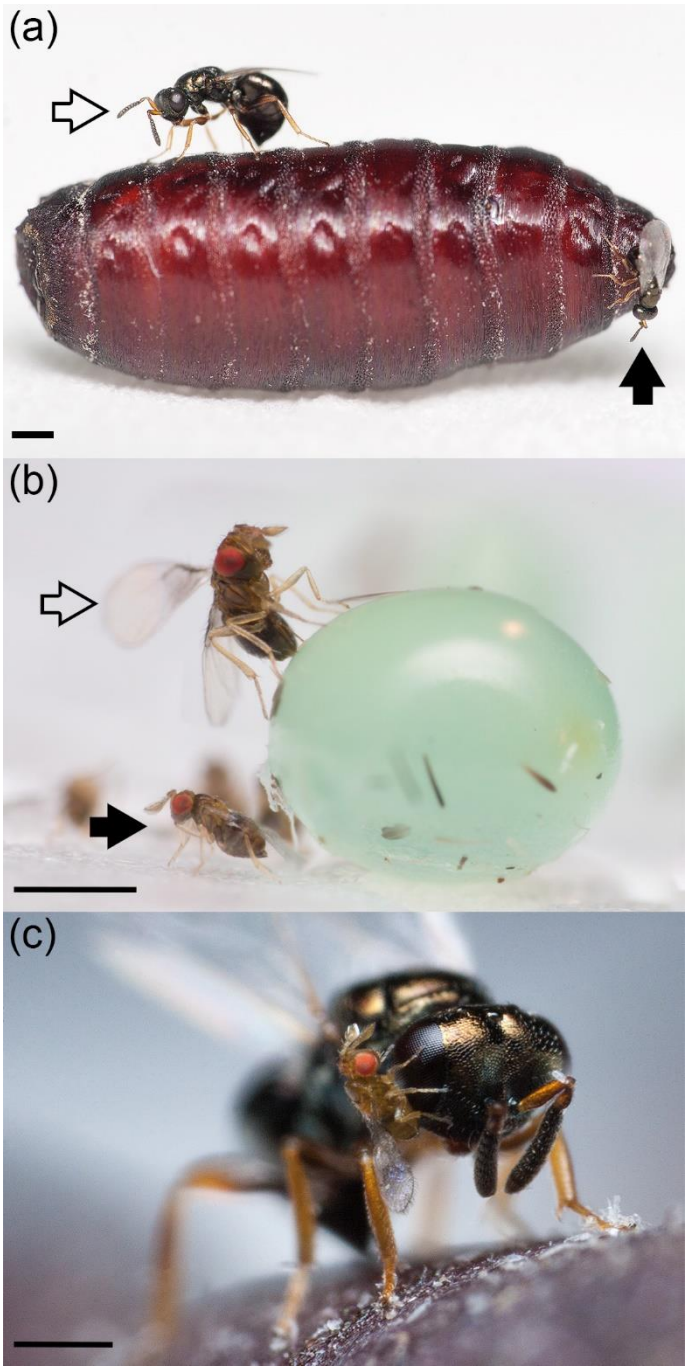
Figure 2. Brain- and body-size scaling in *T. evanescens* and *N. vitripennis*. **(a)** Brain-body size scaling is isometric in *T. evanescens* (red dots, data from Van der Woude et al., 2013), and diphasic in *N. vitripennis* with isometry in small individuals and negatively allometric in large individuals (blue dots, data from Groothuis and Smid, 2017). Red blocks indicate the estimated correspondence to the size classes in the present study, based on body length measurements in Van der Woude et al. (2013) and Groothuis and Smid (2017). Note that the largest body lengths of *T. evanescens* in the present study exceed the measured body lengths in Van der Woude et al. (2013), due to the use of larger host species. Body volume data from Van der Woude et al. (2013) were converted to dry body weights under the assumption of a density of 0.24 g/ml (Kühsel et al., 2017). **(b)** Body length measurements (mean ± SD) of the large and small size classes of *T. evanescens* (red bars) and *N. vitripennis* (blue bars) in the present study. Body length was measured from the head to the tip of the abdomen. Asterisks indicate significant differences based on Welch two-sample t-tests (***, $p<0.001$).

Figure 3. Experimental set-up as used in this study. **(a)** Variation in body size was created by inducing either low (left) or high (right) levels of scramble competition inside the hosts of *N. vitripennis* and *T. evanescens*. For *N. vitripennis,* we varied the ratio between ovipositing females and their *C. vomitoria* host pupae. For *T. evanescens*, we adapted the perceivable host egg surface by partially masking the host eggs with agarose. Females will lay fewer eggs if they perceive a smaller area when drumming on the egg surface with their antennae. **(b)** During conditioning, the wasps experience either an odour (left) or a colour (right) (CS+), while parasitizing a host egg or pupa (US). This is followed by a resting phase in a clean Petri dish or vial on a neutral background (not shown in the figure). Next, the wasps experience the second odour or colour without the presence of the rewarding hosts (CS-). The conditioning procedures are done in a reciprocal manner: half of the groups receive the first odour or colour as CS+ and the other half of the groups receive the second odour or colour as CS+. Small and large wasps are trained simultaneously in separate groups. **(c)** To test memory retention, the wasps are placed in the centre of T-mazes that contain the CS+ and CS- on opposite sides. The number of wasps that make a choice for the CS+ and CS- is recorded. Arrowheads at the olfactory T-mazes indicate an incoming flow of humidified air.

Figure 4. Memory retention over time for small (light bars) and large (dark bars) *T. evanescens* and *N. vitripennis*. Performance index (PI ± SE) shows difference in percentage of preference between reciprocally trained groups. **(a)** Olfactory memory in *N. vitripennis;* **(b)** visual memory in *N. vitripennis*; **(c)** olfactory memory in *T. evanescens;* **(d)** visual memory in *T. evanescens.* Asterisks indicate significant memory retention and differences in memory retention between small and large wasps (Bonferroni-corrected χ^2 pairwise comparisons of GLMM response); *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ns, not significant.

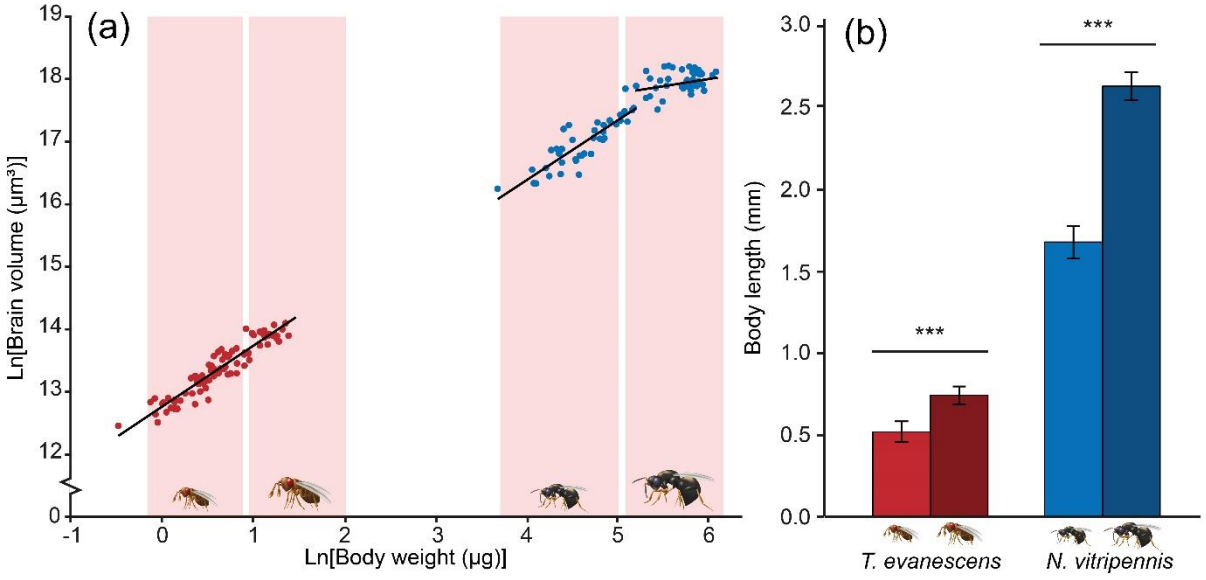
Figures

718 Figure 1



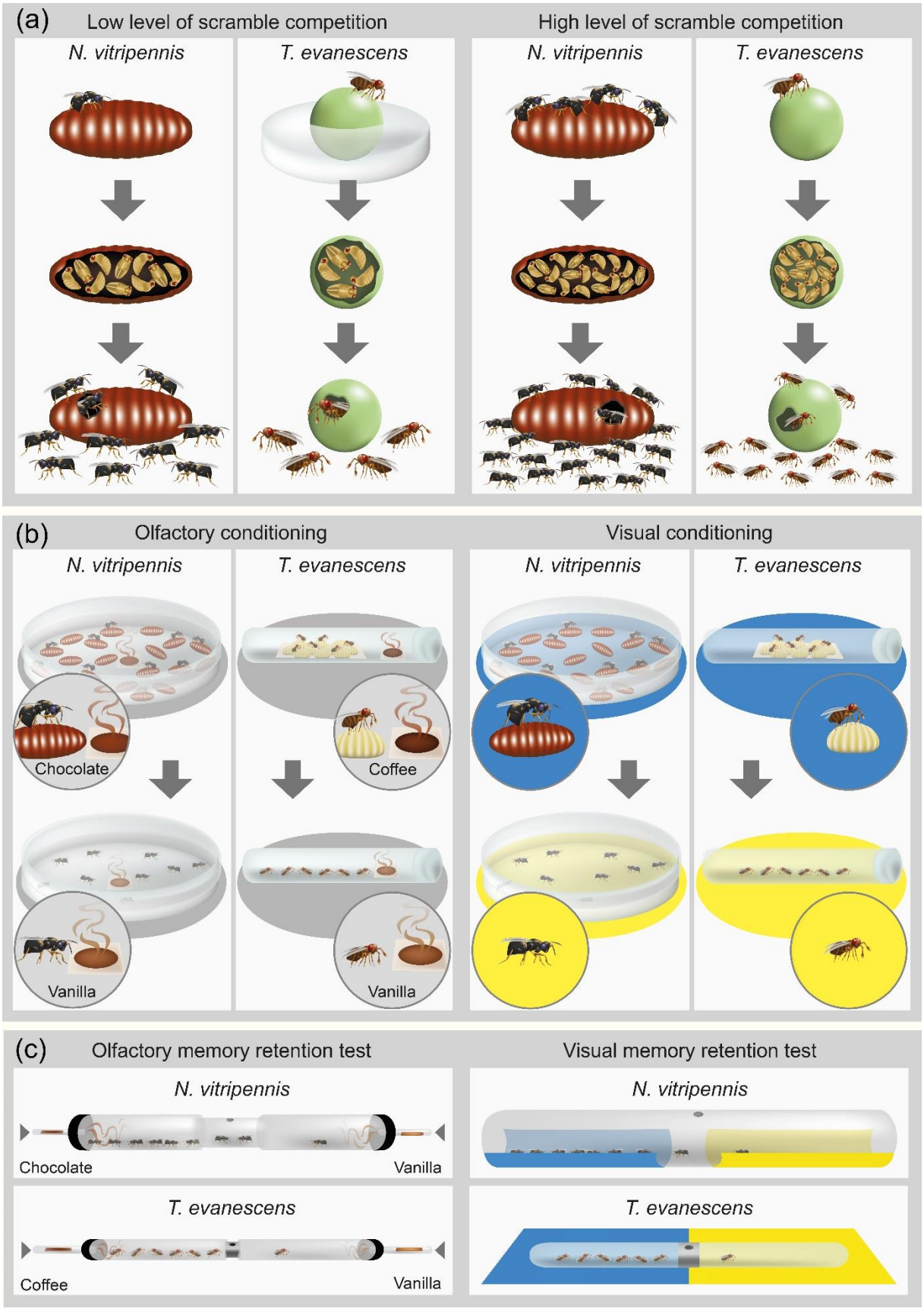
719

720 Figure 2

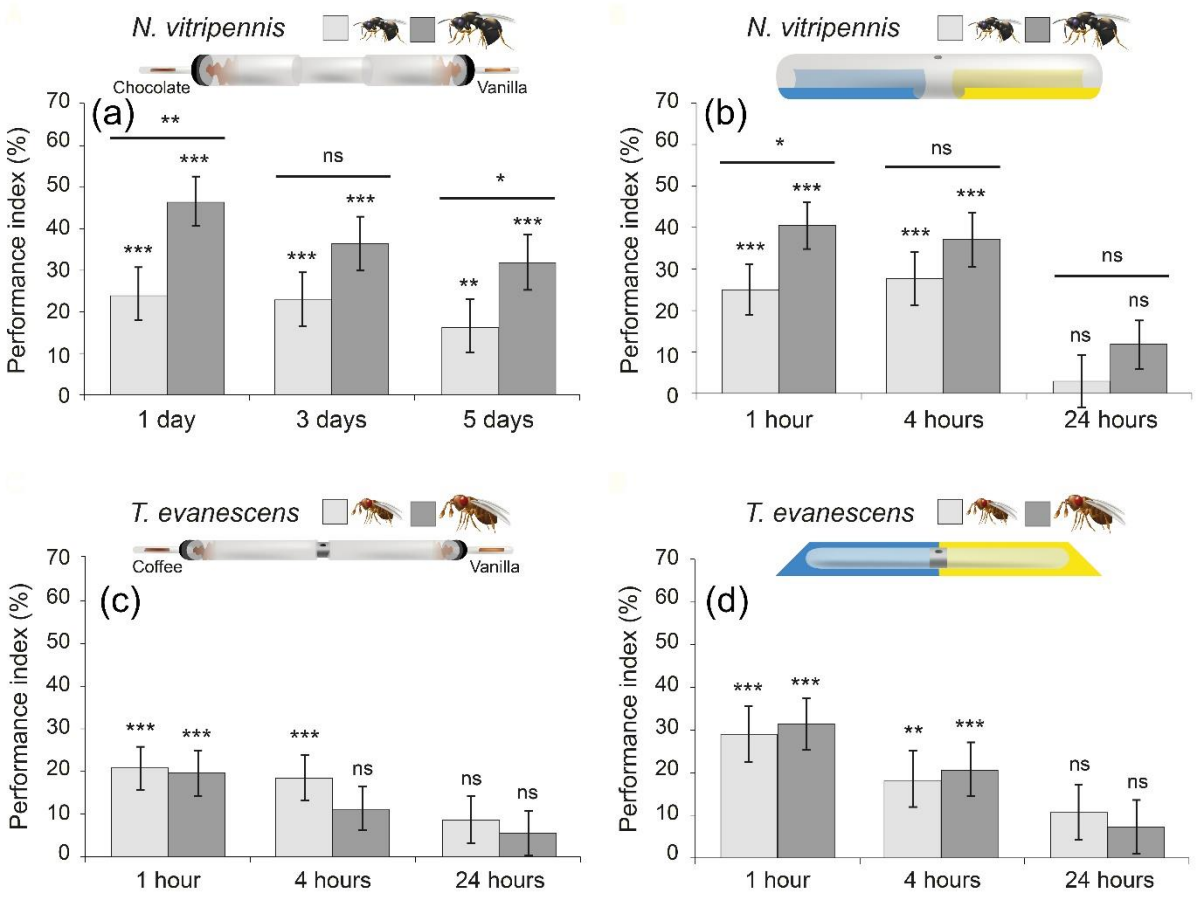


721

Figure 3


(a)
Low level of scramble competition
High level of scramble competition
N. vitripennis
T. evanescens
N. vitripennis
T. evanescens
(b)
Olfactory conditioning
Visual conditioning
N. vitripennis
T. evanescens
N. vitripennis
T. evanescens
Chocolate
Coffee
Vanilla
Vanilla
(c)
Olfactory memory retention test
Visual memory retention test
N. vitripennis
N. vitripennis
Chocolate
Vanilla
T. evanescens
T. evanescens
Coffee
Vanilla

724 Figure 4



725

726

727