Introgression study reveals two quantitative trait loci involved in interspecific variation in memory retention among *Nasonia* wasp species.

K.M. Hoedjes, H.M. Smid, L.E.M. Vet, J.H. Werren

-- Supplementary information--

This supplementary information file contains additional information on (1) the conditioning procedure and memory retention test used in this study, (2) information on the methods used and results from the back-up lines that were generated during the initial introgression experiment, and (3) additional information on the genotyping methodology.

1. Adaptations to the conditioning and memory retention testing procedures that have been described in Hoedjes *et al.* (2012).

The conditioning method as described by Hoedjes *et al.* (2012) was adapted for this study to facilitate conditioning of groups of wasps instead of individual wasps. A group of up to 30-40 wasps was conditioned in a Petri dish (diameter: 90 mm; Greiner Bio-One, Alphen aan de Rijn, The Netherlands) in which 30-40 host pupae were present. Immediately before conditioning, 5 µl of vanilla or chocolate extract (Nielsen-Massey Vanillas Intl., Leeuwarden, The Netherlands) was applied to a piece of filter paper (± 2 cm^2) and placed in the Petri dish. Then the female wasps were released inside the Petri dish and were allowed contact with the hosts for 1 hour. Wasps typically initiate drilling into the host pupae during this period; wasps that did not exhibit this behaviour were carefully removed from the experiment after 30

minutes. After an hour, the wasps were gently taken from the hosts and placed in a rearing vial for 15 minutes, next they were exposed to a second odor, respectively 5 µl chocolate or vanilla extract (CS-), applied to a piece of filter paper which is immediately thereafter inserted in the rearing vial with the wasps, without a reward present for another 15 minutes. When conditioning was finished, wasps were transferred to rearing vials with access to honey and water and kept in a climate cabinet (25^{o}C, 16L:8D photoperiod) until testing. Reciprocal groups of wasps (with either vanilla or chocolate as CS+) were conditioned simultaneously.

Control experiments compared memory retention between individually and group conditioned wasps. Research on *Drosophila melanogaster* has shown that the social environment during conditioning and/or testing can affect the memory scores that are observed (Chabaud *et al.*, 2009; Foucaud *et al.*, 2013). Memory retention of both *N. vitripennis* and *N. giraulti*, which was measured 24 and 48 hours after group conditioning, was comparable to earlier results by Hoedjes *et al*. (2012). In this supplemental experiment we have compared memory retention after group conditioning vs. individual conditioning of *N. vitripennis* 120 (±1) hours after conditioning (Figure S1a). No effect of conditioning procedure ($F_{1,18}$ = 0.97, P = 0.337, n = 10 PIs for both procedures) could be detected. We, therefore, conclude that group conditioning is suitable for this study.

An adaptation was made to the memory retention test during the initial introgression experiment. Memory retention is typically only tested once in each individual, because memory recall (without a reward present) can affect memory dynamics. Exposing animals to the learned cue without a conditioned stimulus, so-called extinction tests, can result in a decay of memory as was shown in the parasitic wasp *Leptopilina boulardi* and *D. melanogaster* (Kaiser *et al.*, 2003; Lagasse *et al.*, 2009). Alternatively, memory can be reconsolidated, depending on the number of extinction tests (Lagasse *et al.*, 2009). The effects of multiple tests on memory are species-specific. During the initial introgression experiment, *Nasonia*

wasps were tested three times in total: once after 24 hours and two times after 72 hours. The aim of this supplementary experiment was to assess if there were any effect of multiple tests on memory retention. Groups of *N. vitripennis* and *N. giraulti* were conditioned and tested as described in the Materials and Methods section. One group of wasps was tested after 24 (±1) hours and once again after 72 (±2) hours (2x tested); a second group was tested once after 72 (±2) hours (1x tested). Both groups were then tested a second time after 72 (±2) hours and compared to a third group of wasps that had not been tested before (control). No effects of multiple tests on memory retention were observed for either species (*N. vitripennis*: $F_{2,27}$ = 1.37, P = 0.280, n = 10 PIs for all test procedures; *N. giraulti*: $F_{2,27}$ = 0.26, P = 0.775, n = 10 PIs for all test procedures) (Figure S1b). This indicates that testing the wasps 3 times during the initial introgression experiment did not have effects on the expected memory retention and this procedure was suitable for selection on memory retention.

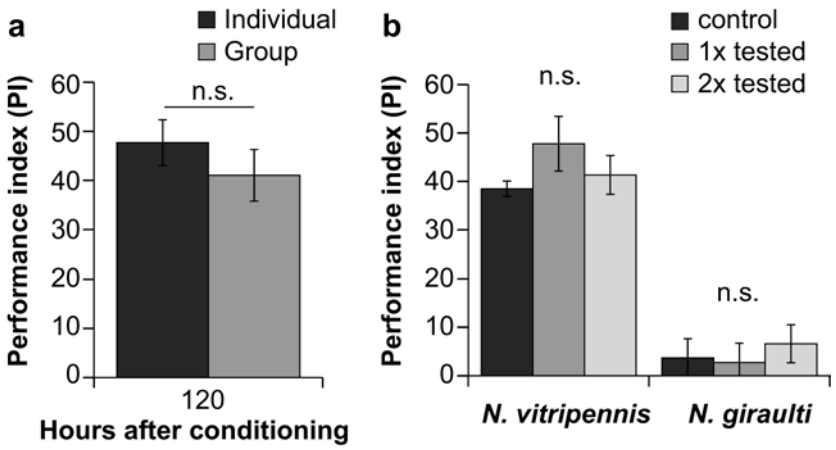

Figure S1: Adaptations to the conditioning procedure and memory retention test. (a) Group conditioning was used in this study instead of individual conditioning. This adaptation did not affect memory retention of *N. vitripennis* when tested 120 hours after conditioning. (b) During the initial introgression experiments, wasps were tested multiple times in order to select for learning rate. Testing for memory retention multiple times was not found to have an effect on memory of *N. vitripennis* and *N. giraulti*.

References:

Chabaud MA, Isabel G, Kaiser L, Preat T (2009). Social facilitation of long-lasting memory retrieval in *Drosophila*. *Curr Biol* **19**: 1654-1659.

Foucaud J, Philippe AS, Moreno C, Mery F (2013). A genetic polymorphism affecting reliance on personal versus public information in a spatial learning task in *Drosophila melanogaster*. *Proc Biol Sci* **280**: 20130588.

Hoedjes KM, Steidle JLM, Werren JH, Vet LEM, Smid HM (2012). High-throughput olfactory conditioning and memory retention test show variation in *Nasonia* parasitic wasps. *Genes Brain Behav* **11**: 879-887.

Kaiser L, Perez Maluf R, Sandoz JC, Pham Delegue MH (2003). Dynamics of odour learning in *Leptopilina boulardi*, a hymenopterous parasitoid. *Anim Behav* **66**: 1077-1084.

Lagasse F, Devaud J-M, Mery F (2009). A switch from cycloheximide-resistant consolidated memory to cycloheximide-sensitive reconsolidation and extinction in *Drosophila*. *J Neurosci* **29**: 2225-2230.

2. Initial introgression experiment (Backup lines BC4-BC6).

In addition to the initial introgression experiment as described in this paper, a back-up was created during the 3rd generation of backcrossing in order to ensure continuation of the project during transition from the laboratory in the USA to the Netherlands. Individuals that had descended from similar females in the BC1 were kept together, resulting in 4 lines selected for a low learning rate and three control lines. Sibmating was allowed in these lines in order to maintain genomic regions involved in regulation of memory retention. Selection for short memory retention was continued, but memory retention was only tested twice 60-72 hours after conditioning. Selection continued up to the 6th generation as described earlier, after

which diapause was induced. In addition to the control lines that were established in the BC1 generation, as described in the materials and methods section, new control lines were established in the 4th and 5th generation by selecting females that chose the learned odour twice from the short memory retention lines (indicated as long memory retention lines). This was done to confirm that the memory retention phenotype of *N. vitripennis* could still be selected for. Univariate ANOVA was used to test for variation in memory retention between control lines and short memory retention lines and a Tukey-HSD post-hoc test was used when appropriate (SPSS version 19; IBM, Armonk, NY, USA).

Memory was decreased when measured after 72 hours in the short memory retention introgression lines compared to the control lines in the 4th generation of backcrossing ($F_{1,26}$ = 8.14, P = 0.008, short memory: n = 16 PIs, control: n = 12 PIs) (Figure S2a). In the 5th generation short memory retention introgression line was compared to the control (BC1) and the newly created long memory retention lines (BC4) (Figure S2b). There was significant variation in memory measured after 72 hours among these lines ($F_{2,89}$ = 3.41, P = 0.038, short memory: n = 42 PIs, long memory (F5): n = 32 PIs, control (F2): n = 18 PIs). The short memory retention introgression lines had decreased memory retention compared to the two other lines, although not significantly (Tukey-HSD: short memory vs. control (BC1) = 0.067, short memory vs. long memory (BC4) = 0.111, control (BC1) vs. long memory (BC4) = 0.848). In the 6th and final generation of selection, 72-hour-memory also differed among lines ($F_{3,119}$ = 4.17, P = 0.008, short memory: n = 38 PIs, long memory (BC5): n = 34 PIs, long memory (BC4): n = 39 PIs, control (BC1): n = 12 PIs) (Figure S2c). Pairwise comparisons revealed a significant difference in memory retention between the short memory lines and the long memory lines (BC4) (Tukey-HSD: short memory vs. long memory (BC4) = 0.004). These results demonstrate that selection for decreased memory retention was successful up to at least 7 generations. Selection for long memory retention, the phenotype of *N. vitripennis*, is

still possible from the introgression lines which were selected for decreased memory retention for 4 to 6 generations, suggesting that this phenotype is likely controlled by genetic factors and not only epigenetic factors.

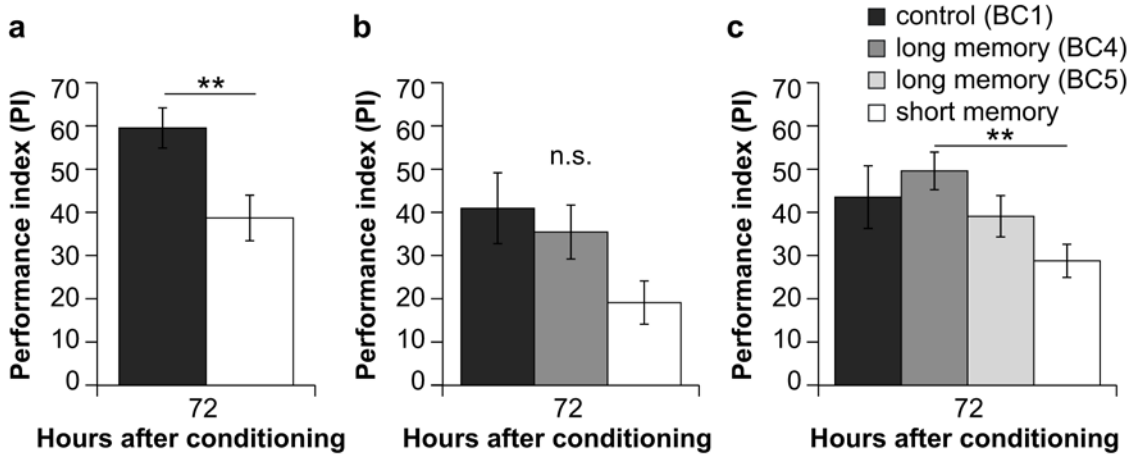

Figure S2 Initial introgression of memory retention: back-up lines. Introgression of memory retention was successfully continued during the (a) 4th, (b) 5th, and (c) 6th generation of backcrossing.

3. Genotyping using a genotyping microarray and primers surrounding an indel-marker in a PCR.

DNA was extracted individually from all wasps using the Gentra Puregene Cell kit (Qiagen, Antwerp, Belgium) following the protocol for a single Drosophila fly. When preparing samples for analysis by genotyping microarray, 2 µl of the DNA from each wasp of a sample was mixed and amplified using the GenomiPhi DNA amplification kit (Sigma-Aldrich, Zwijndrecht, The Netherlands) according to instructions of the manufacturer. DNA was then labelled and hybridized according to Roche NimbleGen's User's Guide and a bulk segregant

analysis was performed as described by Desjardins *et al.* (2013) in order to determine which genomic regions were heterozygous for *N. vitripennis* and *N. giraulti*. This analysis determines the proportion of *N. vitripennis* DNA in a sample for each marker (a score of 1 represents 100% *N. vitripennis*, a score of 0 represents 100% *N. giraulti*). Two samples of F1 hybrids were analysed to determine the suitability of each marker in this analysis. Markers that scored >0.9 or <0.1 were considered unsuitable for bulk analysis and were therefore removed; 14949 of 15546 markers were considered suitable. The average proportion of *N. vitripennis* DNA was determined per 50 subsequent markers. When this average was lower than 0.8, an introgressed genomic region of *N. giraulti* was considered to be present. The scores of individual markers were then inspected manually to determine the boundaries of the introgressed genomic region. The genotype of individual wasps was confirmed using indel-markers within observed introgressed regions in a polymerase chain reaction (PCR) using GoTaq Flexi polymerase (Promega, Leiden, The Netherlands) and primers that surround an insertion-deletion polymorphism between *N. vitripennis* and *N. giraulti* (Table S1). These primers allow distinguishing between *N. vitripennis* and *N. giraulti* based on the size of the amplicon. This method and these primers were also used to genotype individual wasps during the experiment 'Confirmation of memory retention QTLs by independent introgressions'. Table S1 provides details on the sets of primers that were used. The location of each primer set is given as marker cluster (based on the genetic map by Desjardins *et al.* (2013), the location on the chromosome in centimorgan (cM), and the scaffold and base pair position of the 5' base of each forward primer in *N. vitripennis* genome assembly v1.0. All primer sets are suitable when using touch-down PCR conditions: 94^{o}C for 3 min., 9 touch-down cycles in which the annealing temperature drops 1^{o}C per cycle (94^{o}C for 15 sec., 63 - 55^{o}C for 30 sec., 68^{o}C for 1 min.), 28 cycles of (94^{o}C for 15 sec., 55^{o}C for 30 sec., 68^{o}C for 1 min)., 68^{o}C for 6 min. Primers set that have an asterisk following their name also work well using a regular

PCR protocol: 94^{o}C for 3 min., 35 cycles of (94^{o}C for 15 sec., 60^{o}C for 30 sec., 68^{o}C for 1 min)., 68^{o}C for 6 min. Genotype was determined based on size differences between amplicons as visualized on a 1.5% agarose gel.

References:

Desjardins CA, Gadau J, Lopez JA, Niehuis O, Avery AR, Loehlin DW, *et al*. (2013). Fine-scale mapping of the *Nasonia* genome to chromosomes using a high-density genotyping microarray. *G3* **3**: 205-215.

Table S1: genotyping primers

Name	Forward primer	Reverse primer	Marker cluster	Location (cM)	Location (Scaffold)
P1.3*	CGAAATGAGGCTCTACTCGCGCG	GAGAGTATTTATGCACTCGCGCGTG	1.051	Chr1: 45.3	S12-2216571
P1.1*	CGCTTCTACGAACGCGCGGCT	GTGCTCGGCGCATGCAAAACTCG	1.054	Chr1: 47.5	S36-405485
P1.2*	CTGATGCTCCGCGAGGAAAAATCCG	AGAGCGGCAACAGGTGGCGAC	1.054	Chr1: 47.5	S39-358302
P2.9	TGATGCTTTCGACAACATTTCCCCTATCTG	AGCGCACACGATCGCCCTCG	1.057	Chr1: 49.6	S25-258752
P2.5	GCTTCGCTGGCCCGCTATCAG	GCGCCACCGAAAGCCCTCAAC	1.059	Chr1: 51.5	S58-528284
P2.7	CAGCGTCCTGCTCATGTAGCAGC	GCAGGTGAGGTAATTCGCTTGACCG	1.061	Chr1: 52.6	S33-562435
P2.6	GCTGCGCCGTGTCCTCTGTTG	TCGAACTGTATCACACCGCGCACG	1.064	Chr1: 54.8	S1-172028
P2.10	ATATATAATCGGAATGGTCGGACGAGTCG	GATGTTCTCCGCGGACACGCTG	1.064	Chr1: 54.8	S1-896764
P2.8	CGGCAATCACTCGCGAATTTTCGTCC	TGCCACCAGGTTGCAGCCTCAC	1.068	Chr1: 57.7	S1-1267660
P2.4	CAGTGCCGGGAGCAATAACGCG	TGGCAATGGCACGAGGGACTAACAAG	1.071	Chr1: 59.9	S1-1646291
P2.3*	CGCAAAATATAAGACGGATCGGAAGCTCG	CCGACTTAATTGCTGAGATATAATGCGCGC	1.072	Chr1: 60.6	S1-1809330
P2.11	CCGTTTCTCTTAGGCGCGGTATCG	GGAACTCGCTCTCGAGGACGAAC	1.077	Chr1: 66.4	S1-2498836
P2.12	CGGGAAAATTCGCGCGAGAAACAGAC	GCGCTGAGTACACAGAGACGGC	1.084	Chr1: 71.5	S1-2955842
P2.13	GATGGAGTGGCTCTCGGATGACG	CTTGCCTTTCATATTTCATTTCGGCGTATG	1.096	Chr1: 83.2	S1-4580769
P3.1*	CGAGAGACAAGATTTCACGAATACGCAC	ATCACACGCGTCCAATGCGGATGAC	3.000	Chr3: 0.0	S18-59272
P3.2*	ATAAGCGCGGCGACTCCTTCGC	AACGCGCGTACGCAGCCTCC	3.003	Chr3: 2.2	S18-1286317
P3.3	TCCACGACATCCGGCATCGGGATG	GGATTTCACGCTCCGCATTCCGTTG	3.010	Chr3: 8.0	S18-2476428
P4.4	TGCACCCACCCCCACCAATGCTG	CACGTCCGCCCCACTCCACTTG	3.015	Chr3: 11.7	S42-296160
P4.5*	ACTACTGGCTCGCGCGCATTATATAACG	CGACGGGATGGAAAAAAGGGAAATTCAGC	3.017	Chr3: 13.9	S42-504548
P4.6*	CGAACAACATCGCACGCAGCGGAG	GTATTTCCCCGTCGTCGTCGTCG	3.026	Chr3: 22.6	S6-4050087
P4.3	GTCGGCGCGTTAGTGGCGTC	GTAAACCCAATATCGCGTGCAGCG	3.030	Chr3: 25.6	S6-3238822
P4.2*	AGCTTTTGTGCGACGCTTCCGGG	CACGAGCAAACAGGACGCGAGATC	3.033	Chr3: 27.7	S6-2542054
P4.1*	CGGCCCCGACTTTCACCGGC	TTCGAAAAACCAGCCGCAACAGTCG	3.035	Chr3: 29.2	S6-2203321
P9.2*	GCCTCGCAGCGCATAATTTGCCG	CGACGCTCAAGGCCCAAGGC	3.044	Chr3: 37.2	S44-493547
P9.1*	GTCACGAGCAGTGGGTCCGC	CGTGAGCGCGGAGGAAGATCG	3.045	Chr3: 38.0	S111-184751
P9.4*	GCGCGGGGCACTACGCTTTAGG	TATCGCCGAAATAAGGCCAGGCTGAC	3.056	Chr3: 46.0	S22-1568936
P9.3*	ACGGTATCGGATCTCCGGGCTAG	AACGAGGCTGTTTTGACAAGTGTACGCG	3.064	Chr3: 51.8	S22-2444096
P5.1*	GCGGGCTTCGAGTTCAGCGC	CACGCGCGTCTTTGATCTTCCGC	4.005	Chr4: 4.4	S4-4054019
P6.3*	CTTGCCGGCTCATCCGTCCC	CCGCGGGCAGCTGTGTGGTA	4.101	Chr4: 87.6	S9-630535
P6.1*	GTGCGCGACGACGCTCGCCT	TCTTCCCGGGCAACACCCCAC	4.102	Chr4: 89.1	S9-533095
P6.2*	TAATAATCGCGCACTGTCTTCGCCG	GAGTTTCGGTCCGACGCGCC	4.103	Chr4: 90.6	S9-214756
P7.1*	GGCGAGTCGAGAACGGCGCG	GCGAGGAGCAAAGGGTTACATTAGG	5.001	Chr5: 1.5	S14-646706
P7.4	GTCTCTGCATTTAAATCGCCCATCGAGC	GGCCGACTACGCCAGCGATATAC	5.003	Chr5: 2.9	S14-1001415
P7.3	TCATTGCTAACTTTCTAAAGCCGCGTAAGC	GCGAATTGTTTTGACTCGCGGGATTACG	5.003	Chr5: 2.9	S14-1177137
P8.5*	GTGAACGGATAACATTGATCGCAGCCG	AGATGCGATCGCACACGCGCTG	5.017	Chr5: 16.1	S7-2590432
P8.1*	ATGCTGCTGCTGTCCGTGGTGC	CCTGACTCAGTCGGCGTGCG	5.044	Chr5: 36.5	S27-21720
P8.3*	GACCTTCGCCGCAGCTATGTGC	GATACCGGCCACTTCTCCCCC	5.052	Chr5: 41.6	S10-2050116
P8.4	CCGCCCCGAGTTGCAGCACG	GCTTTTTCCTCAAAACTTCCCCGGC	5.075	Chr5: 59.9	S2-2343256