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Supplemental Data

Quantitative mapping of a digenic behavioral trait implicates globin variation in *C. elegans* sensory behaviors

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Supplementary Fig. 1 – Turning rate changes to O₂ and CO₂ increases and decreases. Each strain was tested in response to six step changes, as shown in Fig. 1C, D, and turning rate changes were calculated as in Fig. 1E. Turning responses to simultaneous changes in O₂ and CO₂ (dark and light green bars) repeat those in the manuscript. Complete data and statistics are in Supplementary Table 1, 2.
Supplementary Information

Strain List

CX10774 kyIR1(V,CB4856>N2) V
QX1155 qqIR1(X,CB4856>N2) X
CX10776 kyIR1(V,CB4856>N2) V; qqIR1(X,CB4856>N2) X
CX10860 qqIR1(X,CB4856>N2) X; kyEx2800 [Pnpr-1::npr-1(215V)::sl2gfp Pelt-2::mCherry]
CX11038 qqIR1(X,CB4856>N2) X; kyEx2892 [Pnpr-1::npr-1(215F)::sl2gfp Pelt-2::mCherry]
DA609 npr-1(ad609) X
CX10872 qqIR1(X,CB4856>N2) X; kyEx2802 [Pglb-5::glb-5(HW)::sl2gfp Pelt-2::mCherry]
CX10873 qqIR1(X,CB4856>N2) X; kyEx2803 [Pglb-5::glb-5(HW)::sl2gfp Pelt-2::mCherry]
CX10874 qqIR1(X,CB4856>N2) X; kyEx2804 [Pglb-5::glb-5(N2)::sl2gfp Pelt-2::mCherry]
CX10875 qqIR1(X,CB4856>N2) X; kyEx2805 [Pglb-5::glb-5(N2)::sl2gfp Pelt-2::mCherry]
CX10840 qqIR1(X,CB4856>N2) X; kyEx2791 [Pgcy-36::glb-5(HW)::sl2gfp Pelt-2::mCherry]
CX10779 kyIR1(V,CB4856>N2) V; qqIR1(X,CB4856>N2) qal2241 X
CX10780 kyIR1(V,CB4856>N2) V; qal2241 X
CX7102 qal2241 [Pgcy-35::GFP Pgcy-36::egl-1 lin15+] X
CX11029 qqIR1(X,CB4856>N2) X; kyEx2891 [Pgcy-36::gfp-glb-5(HW) Pelt-2::mCherry]
CX10861 kyIR1(V,CB4856>N2) V; npr-1(ad609) X
CX7376 kyls511 [Pgcy-36::GCaMP Punc-122::gfp] V
CX11035 kyls511 kyIR1(V>CB4856,N2) V; qqIR1(X>CB4856,N2) X
CX11036 kyls511 V; qqIR1(X>CB4856,N2) X
CX11037 kyls511 kyIR1(V>CB4856,N2) V

Wild strains are listed in Supplementary Table 3.

RIAILs used in the study:

QX100, QX102, QX110, QX114, QX115, QX121, QX17, QX181, QX218, QX30, QX49, QX51, QX52, QX53, QX54, QX55, QX56, QX6, QX70, QX71, QX74, QX76, QX78, QX80, QX81, QX83, QX84, QX85, QX90, QX94, QX96, QX97, QX99, QX104, QX11, QX111, QX113, QX116, QX117, QX12, QX182, QX208, QX34, QX37, QX42, QX45, QX61, QX75, QX98, QX103, QX112, QX120, QX129, QX15, QX16, QX3, QX33, QX43, QX44, QX57, QX72, QX82, QX87, QX93, QX95, QX13, QX164, QX172, QX198, QX235, QX237, QX24, QX39, QX60, QX64, QX65, QX86, QX89

Quantitative analysis of RIAIL data

The 78 RIAILs were inbred after a ten generation intercross employing random pair mating with equal contributions of each pair to each generation. Because of population size attrition during the intercross phase, due to failed crosses and segregating mortality and sterility, two separate inbred lines were derived from hermaphrodites in the tenth generation of the cross. The resulting RIAILs include pairs of lines that exhibit elevated relatedness; the lines are largely unique because of the random allelic fixation and breakpoint accumulation during the inbreeding phase of RIAIL construction. Nevertheless, to avoid artefactually high lod scores and low P-values from the background relatedness among these lines, we divided the dataset into two subsets, with each pair of related strains split between the subsets. Linkage scans were performed separately for the two subsets and lod scores were then summed, and p-values were estimated from 10,000 permutations performed separately for the two subsets.
Given the presence of selection during the RIAIL construction (Seidel et al., 2008), there is no analytical method available to convert RIAIL recombination fractions into meiotic recombination rates. However, our high marker density renders the exact form of map function irrelevant. Three markers that did not vary within one of the subsets were excluded, leaving 1452 markers. QTLs were incorporated into additional rounds of interval mapping as covariates in parametric analysis under a normal model, and no additional QTLs were found. The fraction of variance explained by each QTL and their interactions were estimated using ANOVA. The structured nonparametric linkage scan for the O$_2$ increase/CO$_2$ decrease trait yielded two peaks on the X chromosome, the peak at npr-1 (lod score 5.2), coincident with the QTL from the O$_2$ decrease/CO$_2$ increase trait, and a peak 3 Mb to the right, at 7.7 Mb (lod score 5.4). However, incorporation of the npr-1 genotype as a covariate in the analysis eliminates the peak at 7.7 Mb, suggesting that this second peak is attributable to noise whose baseline is elevated by linkage to npr-1. Similarly, incorporation of glb-5 genotype as a covariate causes the X-linked QTL to localize at the npr-1 peak.

**Generation of introgression lines**

To generate the introgression line containing the HW allele of glb-5 in an N2 background, CB4856 was crossed to BC277 (unc-46(e177) dpy-11(e224) V) (two mutations that flank glb-5) and Dpy nonUnc F3 progeny homozygous for the HW allele of glb-5 were selected. These were backcrossed to N2 for 10 generations selecting for Dpy animals before removing the dpy-11(e224) allele by mating to N2 and selecting non-Dpy F2 animals homozygous for the HW allele of glb-5. This introgression was given the name kylIR1(V,CB4856,N2). To generate QX1155, the introgression line containing npr-1(215F) in an N2 background, we began with QX108, a RIAIL with npr-1(215F) at position 4,768,758 and the N2 allele at the next marker to the right, pkP6106 at 4,892,213. We crossed lon-2(e678) males to QX108 and then crossed the hermaphrodite offspring to lon-2(e678) males for 20 generations, selecting nonLon hermaphrodites each generation.

The left recombination breakpoint of kylIR1 on V is between markers at 5,134,620 (haw76206) and 5,369,558 (pkP5110); the right breakpoint is between the duplication in glb-5 (~5,562,500) and a marker at 5,573,241 (snp_Y61A9L[1]). The left recombination breakpoint of qylIR1 on X is between markers at 2,550,901 and 3,157,209 (pkP6145); the right breakpoint is between npr-1(215F) (~4,769,500) and pkP6106 (4,892,211).

**Isolation of wild strains**

Compost was collected from a bin at 1464 Hopkins St., Berkeley, on 24 November 2007, and soil was collected from under rotting fruit in a backyard garden at 363 Jersey St., San Francisco, on 26 November 2007. The material was spread on NGM plates seeded with OP50 on 27 November. Individual dauer larvae were isolated as they emerged from the compost sample (QX1217-QX1233). The sample from San Francisco yielded six young adult hermaphrodites on 29 November (QX1211-QX1216). After each animal had laid eggs, we removed and lysed the parent for genotyping. All strains had HW npr-1 and glb-5 genotype. The npr-1 sequences revealed two additional SNPs in two of the San Francisco strains, indicating that this collection includes at least two different genotypes. The Berkeley npr-1 sequences are all identical.

**Quantitative analysis of behavioral data**
A one-hour long turning assay of 20-30 animals consisted of ten six-minute cycles between two gas mixtures, with three minutes of each gas mixture in each cycle. For each assay, the first six-minute cycle between gas mixtures was discarded, leaving nine identical six minute intervals that were averaged together. Each recording was binned into 20 second windows. Data for all strains are included in Supplementary Table 1. To create data for changes in turning rate, average turning rates for 80 seconds prior to a shift in gas concentration were subtracted from the average turning rate 20–60 seconds after each shift. Averages and standard errors represent at least 3 experiments for each strain and condition, except for Fig. 1F, in which the final index for each RIAIL was averaged from two separate experiments.

Aggregation and bordering assays were scored one hour after placing sixty young adult animals on a four-day old lawn of OP50 bacteria. Each strain was analyzed at least three times.

Statistics were calculated using GraphPad Prism software, using either the unpaired two-tailed Student’s t-test or 1-way ANOVA with the Tukey correction for multiple comparisons between strains, as appropriate. To determine if CO₂ downshifts interacted with O₂ upshifts, multivariate linear regression was run in R to determine if an interaction term was significant. Full statistics are included in Supplementary Table 2.

Molecular biology of glb-5

glb-5 cDNAs were isolated by RT-PCR using the primers 5’-aaccttgctagcatgcagacgacagtctggag-3’ and 5’-aaccttggtacctcaaatctcagatttgcagtaaccttg-3’. The primers amplified a single ~1200 bp band from HW RNA and a single ~1400 bp band from N2 RNA, which were cloned into the Nhel/Kpnl sites of pSM-SL2GFP. cDNA sequences from three different clones from each genotype were identical. The glb-5 cDNA sequence from HW was:

```
atgaacgagacgcagctggagttgaaataattgtatgaagagtttccgaaataccacagctttggaatgtaaacttaactactgttatgaaataggtgcaaaaccatactaggaatatctctaaaggaagccagactgacctttgaaatgtaaacttaactactgttatcgaaaaa
```

The sequence of the glb-5 cDNA isolated from N2 was (duplication bolded):

```
atgaacgagacgcagctggagttgaaataattgtatgaagagtttccgaaataccacagctttggaatgtaaacttaactactgttatgaaataggtgcaaaaccatactaggaatatctctaaaggaagccagactgacctttgaaatgtaaacttaactactgttatcgaaaaa
```

The sequence of the glb-5 cDNA isolated from N2 was (duplication bolded):

```
atgaacgagacgcagctggagttgaaataattgtatgaagagtttccgaaataccacagctttggaatgtaaacttaactactgttatgaaataggtgcaaaaccatactaggaatatctctaaaggaagccagactgacctttgaaatgtaaacttaactactgtatatcgaaaaa
```

The sequence of the glb-5 cDNA isolated from N2 was (duplication bolded):

```
atgaacgagacgcagctggagttgaaataattgtatgaagagtttccgaaataccacagctttggaatgtaaacttaactactgttatgaaataggtgcaaaaccatactaggaatatctctaaaggaagccagactgacctttgaaatgtaaacttaactactgtatatcgaaaaa
```

The sequence of the glb-5 cDNA isolated from N2 was (duplication bolded):

```
atgaacgagacgcagctggagttgaaataattgtatgaagagtttccgaaataccacagctttggaatgtaaacttaactactgttatgaaataggtgcaaaaccatactaggaatatctctaaaggaagccagactgacctttgaaatgtaaacttaactactgtatatcgaaaaa
```
The presence of the \textit{glb-5} exon duplication was assayed using three-primer PCR. In the absence of the duplication, primers \textit{globX\_for} and \textit{globX\_rev2} amplify an 1159 bp fragment. In the presence of the duplication, \textit{globX\_for} and \textit{globX\_rev} amplify an additional 684 bp fragment. \textit{npr-1}(g320) was genotyped by amplifying a fragment with primers \textit{sjj\_C39E6.6\_f} and \textit{sjj\_C39E6.6\_b} and sequencing the products.

**Plasmid construction**

The \textit{npr-1} cDNA, including 5' and 3' UTRs sequences, was amplified by RT-PCR from \textit{C. elegans} mixed stage RNA using the oligonucleotides 5'-agctacctgctagcacatatggcag, tccgtttccttaattcgca and cloned into the vector pSM-SL2GFP via the NheI and KpnI sites. To generate the \textit{npr-1}(215F) allele, the QuikChange® Site-Directed Mutagenesis Kit (Stratagene) was used to change the 215\textsuperscript{th} Valine to a Phenylalanine using primers 5'-ggccttctgctatgcataattttttcggtgctcagcaagcgtg and 5'-cacgcttgtgcagcagcaagcaatatggctagccagagcc-3'.

Promoters were amplified from N2 genomic lysate, with the exception of the \textit{glb-5} promoter, which was amplified from HW genomic lysate, and cloned into the pSM vector. The sequences of the promoter ends are shown below:

- \textit{glb-5}: atctgtgagttagctatttttag, tecgtttctgaatttagca
- \textit{npr-1}: ctcgagttctgtggctttgttcgtc, etccatgactaaagaaaaatatcagcagagcc-3'
- \textit{gcy-36}: tggatgtttgttagttggtttgga, aaattcaaaacagggtcactcaaca

**Generation of transgenic lines**

Transgenic lines were made by microinjection using standard protocols (Mello et al., 1991). A fluorescent co-injection marker (1 ng/µl of \textit{elt-2::mCherry}) was used to identify transgene-containing animals, and pSM vector was added to make the final injection mix at a concentration of 100 ng/µl. Plasmids with the \textit{glb-5} promoter were injected at 1 ng/µl, \textit{npr-1} promoter at 100 ng/µl, and \textit{gcy-36} promoter at 1 ng/µl. For each transgene tested, a minimum of three lines were initially scored in the coupled O$_2$/CO$_2$ response. One or two representative strains were then scored multiple times in all three conditions.
References
