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Genetic Variation in Neurotransmitter Receptors Generates Behavioral Diversity

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GENETIC VARIATION IN NEUROTRANSMITTER RECEPTORS GENERATES BEHAVIORAL DIVERSITY

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GENETIC VARIATION IN NEUROTRANSMITTER RECEPTORS GENERATES BEHAVIORAL DIVERSITY

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Variation in behavior among individuals is both remarkable and of great significance to society. People differ in locomotor skills, in sleep patterns, in their willingness to take risks, and in how they relate to other people. Whereas diversity enriches society, extreme behavioral deviations can be pathological, so it is important to identify the causes of behavioral variability. It is clear that both the environment and genetics contribute to behavioral diversity in all animals, but the nature of the specific genes involved is only beginning to emerge. The nematode worm *Caenorhabditis elegans* is a good animal model to study the genetic and neuronal bases of behavioral variation, as there are large differences in behavior between naturally-occurring strains, and powerful tools exist to characterize these differences.

One example of the behavioral diversity of *C. elegans* is the existence of different thresholds for exploration–exploitation tradeoffs: some strains decide to exploit resources more thoroughly, while others decide to abandon resources earlier and explore other options. Using quantitative genetic tools I have found that genetic variation in the adrenergic receptor *tyra-3* affects this exploration–

exploitation decision. *tyra-3* responds to the neurotransmitter tyramine, which is related to vertebrate adrenaline and noradrenaline. *tyra-3* modifies the activity of sensory neurons that detect food cues and that regulate the decision to abandon depleting food resources. In strains that are more prone to exploration *tyra-3* is expressed at lower levels, and this altered expression modifies the response of the sensory neurons to food. Variation in a gene that affects the response to the environment helps explain how nature and nurture interact to produce behavioral outcomes.

In addition to variation in exploratory behavior, *C. elegans* strains also differ in social behaviors. In most strains animals aggregate with each other, whereas a few strains have evolved a solitary life-style. Variation in the neuropeptide Y receptor homologue *npr-1* contributes to social behavior variation, but I found that other genes are also involved in this behavior. Through quantitative genetic analysis I identified polymorphisms in the GABA-gated cation channel *exp-1* that generate variation in social behavior.

Based on existing behavioral diversity in *C. elegans*, I discovered genetic variation in two neurotransmitter receptors and characterized the way in which this variation modifies the neuronal circuits that generate behavior. Consistent with findings in other systems, my results suggest that genetic variation in neurotransmitter receptors is a common way of generating behavioral diversity in animals.

In memory of my zeide Sioma, who inspired me to understand how things work and why they are the way they are

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"All animals are equal, but some animals are more equal than others."

–George Orwell

INTRODUCTION: Genetic contributions to behavioral diversity

The word "behavior" refers to all observable actions of animals or people, ranging from simple reflex actions to complex behavioral sequences or patterns. A single behavior represents a combined response to external stimuli, internal motivational states, innate genetic programs, and experience-dependent learning. These four domains emerge from neurobiological systems for sensory processing, emotion and motivation, neuroanatomy, and plasticity, respectively. The neurobiological systems are evolutionarily ancient and substantially shared between humans and other animals, although their action is shaped by higher cognition in humans.

Many single-gene mutations that affect animal behaviors have been identified through classical genetic screens and knockout mutants, and rare single gene mutations affect human behaviors as well. A notable example of the universality and specificity of single behavioral genes is provided by the genes that regulate circadian behaviors, first identified through induced mutations in *Drosophila* and mice [1,2], and then found to be mutated in humans with the rare single gene circadian disorder Advanced Sleep Phase Syndrome [3,4]. Other rare human mutations of large effect can give rise to specific syndromes of

overeating and obesity (leptin receptor) [5], narcolepsy/cataplexy (hypocretin) [6], or mental retardation and developmental delay [7], among other disorders. Despite these powerful examples, however, most common genetic variation in human and animal behavior cannot be explained by known single-gene mutations.

The subject of my thesis is common genetic variation in behavior. Genetic variation plays a role in human personality traits, in common mood disorders, addiction, and anxiety disorders, and in rare neurodevelopmental and psychiatric disorders such as autism and schizophrenia. In humans, however, genetic factors are difficult to disentangle from individual experiences, environments, and choices. Studies of natural behavioral variation in non-human animals have begun to identify the molecules that generate common differences in behaviors ranging from fly locomotion, to nematode decision-making, to rodent emotional and social behaviors. Recent technological advances driven by genome sequencing allow the identification of genetic markers in any species; these methods, coupled to high-throughput genotyping, are facilitating rapid advances in the genetic mapping of normal and pathological behavioral traits.

In this introduction I describe conceptual insights and molecular discoveries from studies of genetic variation within species, and between closely-related species. I first describe the quantitative evidence for genetic effects on behavior and for a complex genetic architecture of most behavioral traits, and then describe representative studies that move from a behavior to

molecules, and back to behavior. Gene-environment interactions are an essential theme of behavioral genetics, and I will use specific examples to show how the gene-environment interface illuminates the nature of behavioral variation. An important question in the field is to understand how the brain translates genetic changes into behavior, and initial examples show the way forward – and have led to new insights into the neurobiological basis of behavior. Finally, I argue that certain classes of genes, including sensory genes and genes that affect neuromodulatory systems, will be disproportionately associated with variation in behavior because of their evolvability. I discuss these ideas in the context of balancing selection that shapes and maintains behavioral variation in nature.

Quantifying genetic contributions to animal and human behavior

Humans have bred domesticated animals for specific behavioral traits for thousands of years, an implicit recognition that such traits are genetically encoded. Domestic dogs provide a familiar example: dogs are calmer and less aggressive than their wolf ancestors and, in addition, different breeds have been bred to excel at working tasks such as herding, retrieving, pointing, and scent tracking [8].

In animals, heritability is typically quantified by regression of offspring trait values against those of their parents. A survey of 57 behavioral traits in animals showed that the average heritability of these traits is 38% [9]. This survey included

behaviors such as courtship in insects, defensive behaviors in snakes, foraging behaviors in flies, snakes, and birds, and learning in insects, mice, pigs, and rats.

Whether human and non-human animal behavior are influenced to the same extent by genetics is an important question. In humans, heritability estimates for nonclinical behaviors including feeding, drug use, and social behaviors range from 15-60% [10-13], and the heritability of stable personality traits such as neuroticism or extraversion is estimated at 30-60% [13]. These results suggest that the overall influence of genes on human behavior and on animal behavior is similar in magnitude.

The strongest evidence for genetic effects in human behavioral variation comes from family and adoption studies demonstrating elevated risk for psychiatric disorders in relatives of patients with those disorders. Consistent with the importance of genetic risk factors, the degree of risk positively correlates with genetic relatedness. Among family studies, the greatest weight has been placed on twin studies that compare monozygotic twins, who share nearly 100% of their genetic constitution, with dizygotic twins or other full siblings, who on average share 50% of their genetic makeup. The monozygotic twin of a schizophrenic patient has about a 50% risk of schizophrenia [14], which represents an enormous increase over the population risk of ~1%; still, the fact that it is less than 100% demonstrates the existence of non-genetic components. By the same formulation, an affected monozygotic twin with a disorder predicts a

60% risk for autism [15,16], and a ~40% risk for bipolar disease [17-19], anxiety disorders [20], or depression [21,22].

A puzzling aspect of these studies appears when the risk to first-degree relatives is examined (dizygotic twins, other siblings, parents, and children of affected individuals). In the common disorders like anxiety and depression, the risk to these individuals is about half that of the monozygotic twin [21,20,22]. This relationship matches theories of additive variation, where multiple alleles act independently of one another to influence risk. In the rare disorders like autism, schizophrenia, and bipolar disorder, however, the risk to first-degree relatives is much lower than half that of the monozygotic twin [15,17-19,14,16]. For these disorders, the genetic risk may reflect new mutations and combinations of risk alleles with nonlinear interactions. In addition, dizygotic twins have a higher risk than other first-degree relatives, an observation that might reflect prenatal environment or other developmental risk factors.

Genome-wide association studies indicate that common genetic variants contribute to the risk of schizophrenia and bipolar disorder, but are less important than they are in other complex non-psychiatric diseases [23-26] (but see also [27] for an alternative viewpoint). In addition, an unknown, but growing, fraction of schizophrenia and autism cases are associated with *de novo* mutations or rare transmitted mutations, often copy number variants (CNVs), that can cause a large increase in risk [28-34]. Each identified high-risk variant is present in at most 1% of patients, indicating that one disease can result from

hundreds of different genetic causes. Adding to this complexity, several SNPs and a rare single-gene risk factor (DISC1) increase risk both for schizophrenia and for bipolar disorder, suggesting that shared genetic factors influence multiple disorders [35,23,24,36]. These results suggest that genetic causality and heterogeneity will challenge the existing classification categories for psychiatric disorders.

Most behavioral traits have a complex genetic basis

The first step towards understanding the genetic basis of behavioral trait variation is to define its genetic architecture – the number, frequency, effect size, dominance relationship, and interactions of genetic variants that affect a trait in populations of a species. For organisms in which crosses can easily be performed, linkage-based mapping techniques such as quantitative trait locus (QTL) mapping are used. In outbred populations such as humans and wild animals, genetic association approaches are more useful if a sufficiently large number of individuals and genetic markers are examined (e.g. genome-wide association studies). In general, mapping methods are similar to those used in non-behavioral traits, but special care must be taken to minimize measurement noise, since behavioral traits are 'noisier' than morphological or physiological traits (Appendix 1).

Classical QTL mapping demonstrates a complex genetic architecture. QTL mapping of progeny from an intercross of two strains measures the correlation between trait values and DNA markers across the genome, and infers the number of loci that affect the trait, their location, and the contribution of each locus to the total trait variance (**Figure 1.1**). QTL analysis between inbred strains has been used to map trait differences in mouse learning, fear, anxiety, circadian rhythm, responses to addictive drugs, and activity levels; rat fear, anxiety, and responses to addictive drugs; and *Drosophila* olfactory behavior, mating behavior, and locomotor reactivity [37-40]. The advantage of QTL mapping using defined crosses is that a sufficiently large collection of F2s or recombinant inbred lines rigorously tests each locus for variation, and also tests combinations of alleles and some genetic interactions (epistasis). The disadvantage is that discovery is limited to the alleles that happen to vary between the two starting strains.

A modified QTL approach directed at capturing broader population variation starts from a pool of parental strains, not just two strains. In *Drosophila,* a collection of wild flies from the Raleigh Farmers' Market has been used to generate 192 inbred lines representing broader genetic variations from wild populations [41]. In mice, a Collaborative Cross has been structured to capture variation from eight different mouse strains in a pool of recombinant inbred lines [42]. The advantage of this approach compared to the classical QTL approach is its genetic breadth; the disadvantage is that any phenotype that

requires a combination of genetic variants will probably not be represented in a realistic number of inbred strains. In all of these examples, the existence of stable inbred strains that can be genotyped once and then tested for many phenotypic traits provides an immense increase in experimental power; such carefully-constructed strains exist for *C. elegans* [43], *Drosophila* [41], and mouse [42].

QTL approaches in rodents and flies unambiguously show that the genetic architecture of behavioral traits is complex. For example, QTL analysis of two inbred mouse strains was conducted for "emotionality," which describes a set of fear- and anxiety-related behavioral responses such as avoidance of exposed areas and inhibition of movement after foot shock. A single pair of mouse strains yielded at least 16 distinct emotionality loci [38]. Moreover, a different set of QTLs was found for the same emotionality behavior in different strains of mouse [38]. Similar results from *Drosophila* locomotor activity and aggression support a similarly complex architecture [40,44]. Importantly, all QTLs do not contribute equally to a phenotype. An analysis of over 200 behavioral QTLs affecting 20 different traits in mice and rats demonstrated that the effect size of QTLs is exponentially distributed: about 10% of the QTL had large individual effects accounting for 10-20% of trait variance and a large number of loci contributed increasingly smaller effects [38,45]. The traits characterized in this analysis included motor activity, learning, emotionality traits, and drug related behaviors.

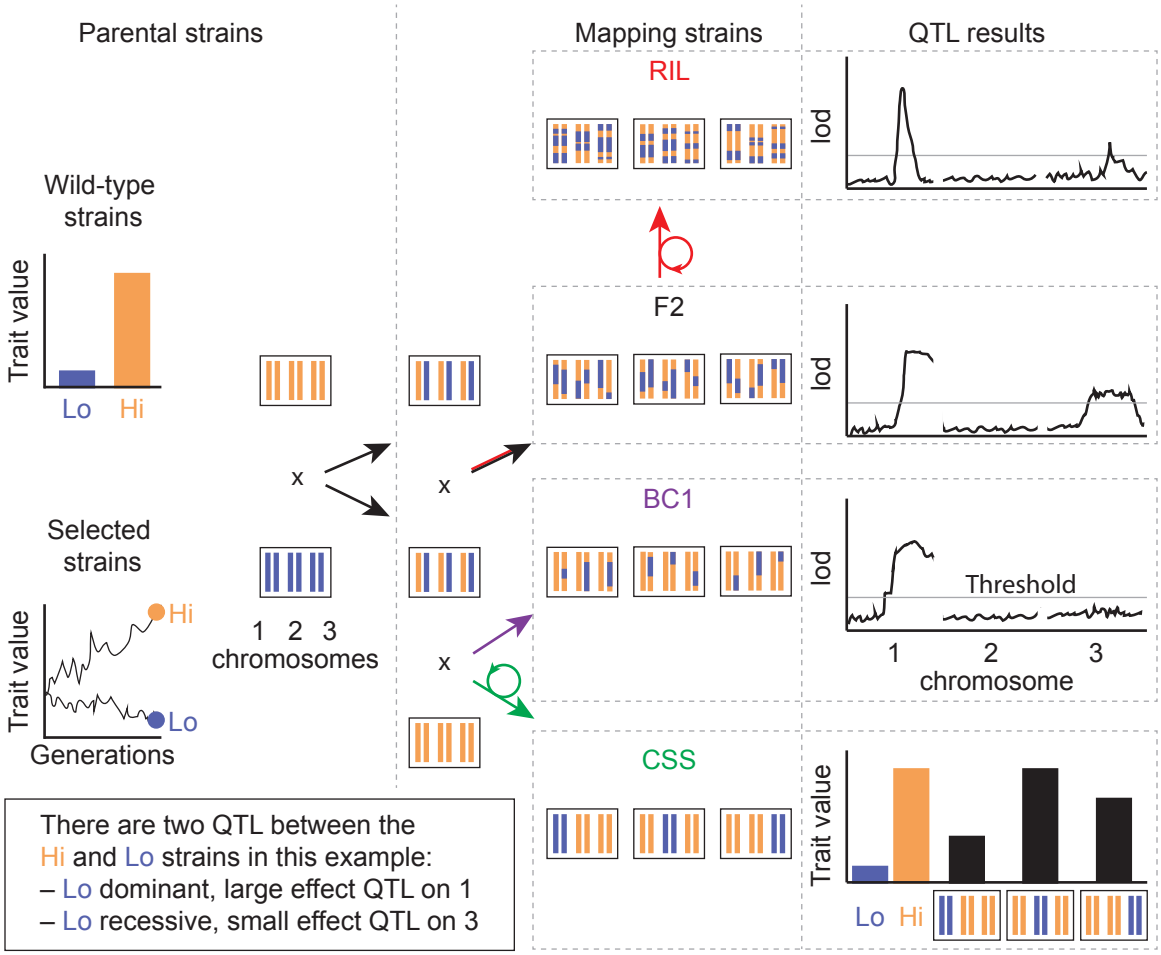

Figure 1.1

Strategies for linkage-based mapping. The parental strains, called Hi and Lo in this example, can be wild-type strains that differ in the trait of interest (upper left) or strains that were selectively bred to differ in the trait, starting from a heterogeneous population (lower left). Three pairs of chromosomes from hypothetical diploid individuals are shown. Arrows with circles indicate repeated crosses of the same type. In this example, there are two additive QTL, one of large effect on chromosome 1, with a dominant Lo allele, and another of small effect on chromosome 3, with a recessive Lo allele. Characterizing recombinant inbred lines (RIL) or F2 identifies both QTL. F2 give information about their dominance, but RIL provide better resolution. Mapping with a backcross (BC1) can only identify QTL that are recessive in the parental strain used for the backcross – Hi in this example. Only three strains for BC1, F2, and RIL are shown, but usually dozens to hundreds are used. lod, logarithm of the odds. Chromosome substitution strains (CSS) identify both QTL but give no indication of their location within the chromosome or their dominance.

Linkage studies with introgression strains show large effects on behaviors. An approach complementary to QTL analysis is to analyze introgression strains that have defined DNA segments from one strain introduced into a different strain background (**Figure 1.1**). The introduced segments can be full chromosomes in chromosome substitution strains (CSS), or smaller chromosomal intervals in congenics. This approach is particularly powerful when the whole genome is covered in a panel of CSS strains or congenic strains; such panels have been developed in *C. elegans* [46,47], in *Drosophila melanogaster* [48], in rats [49], and in mice [50-52] (where congenics in a panel are referred to as genome-tagged mice, or GTM).

CSS and GTM have been used to characterize fear and anxiety-related traits in mice and, like QTL crosses, CSS and GTM indicate that multiple loci contribute to these behavioral traits [53,54]. Individual chromosomes, however, have been demonstrated to have large effects on these behaviors using CSS, sometimes accounting for half of the trait difference between the two parental strains [53] – a greater effect than is inferred for any single QTL identified through classical QTL mapping. Even more remarkably, many chromosomes from a single strain can have large effects such that, in combination, they "account" for more than 100% of the difference between the two starting strains [55]. The larger apparent effect of single QTLs in a CSS is partly due to the statistical structure of the experiment. A QTL cross with multiple loci segregating is used to explain the segregating variance in a trait, which must add up to 100%. By contrast,

studies of CSS describe the effect of a genetic region on mean trait value, which more closely matches the intuitive concept of effect size. In addition, QTL analysis detects the average effect of a variant across many different genetic backgrounds, including those where epistatic interactions obscure the effects of the QTL, whereas chromosome substitution interrogates the variant in a single background. Many genetic effects are background-dependent in model systems; for example, the viability of certain gene knockouts in mice and yeast depends on the strain background [56,57]. Epistatic interactions among behavioral traits are well-recognized. Epistasis between natural QTL has been detected in courtship, foraging, locomotion, learning, and aggressive behaviors in insects [9,58,59,44], and in fear and anxiety traits in mice [54].

Lessons for human behavior. Based on animal studies, we expect any individual human behavioral trait to be affected by many different genetic variants. Across the entire population, individual variants are likely to have small to moderate effect sizes. However, the CSS and GTM results imply that in any one individual – in a single genetic background – a particular genetic variant may have a large effect that is lost by averaging all of the epistatic effects over the entire population. At a practical level, this conclusion suggests that studies *combining* family-based designs with genome-wide association studies or sequencing approaches will help in finding causative genetic variants [60]. Genome-wide association can detect many variants but, like QTL studies, effect

size will be diluted by the heterogeneity of backgrounds in the population. Human families are not as inbred as CSS strains, but they are considerably less heterogeneous in genetic background than whole populations.

Fine-mapping and functional validation of QTL

The best strategy for moving from a QTL to a single gene varies depending on the organism and on the complexity of the trait. Approaches that have been successful for identifying and validating behavioral genes are outlined below.

Introgression strategy. Genes associated with QTLs can be identified by an introgression strategy, in which smaller and smaller regions spanning the QTL are crossed into a recipient genetic background (**Figure 1.2a,b**). Eventually, introgression of a single gene or variant can be shown to affect the recipient strain's behavior. This approach has been especially successful in *Caenorhabditis elegans* [61-63], whose 3-day generation time facilitates multiple rounds of introgression.

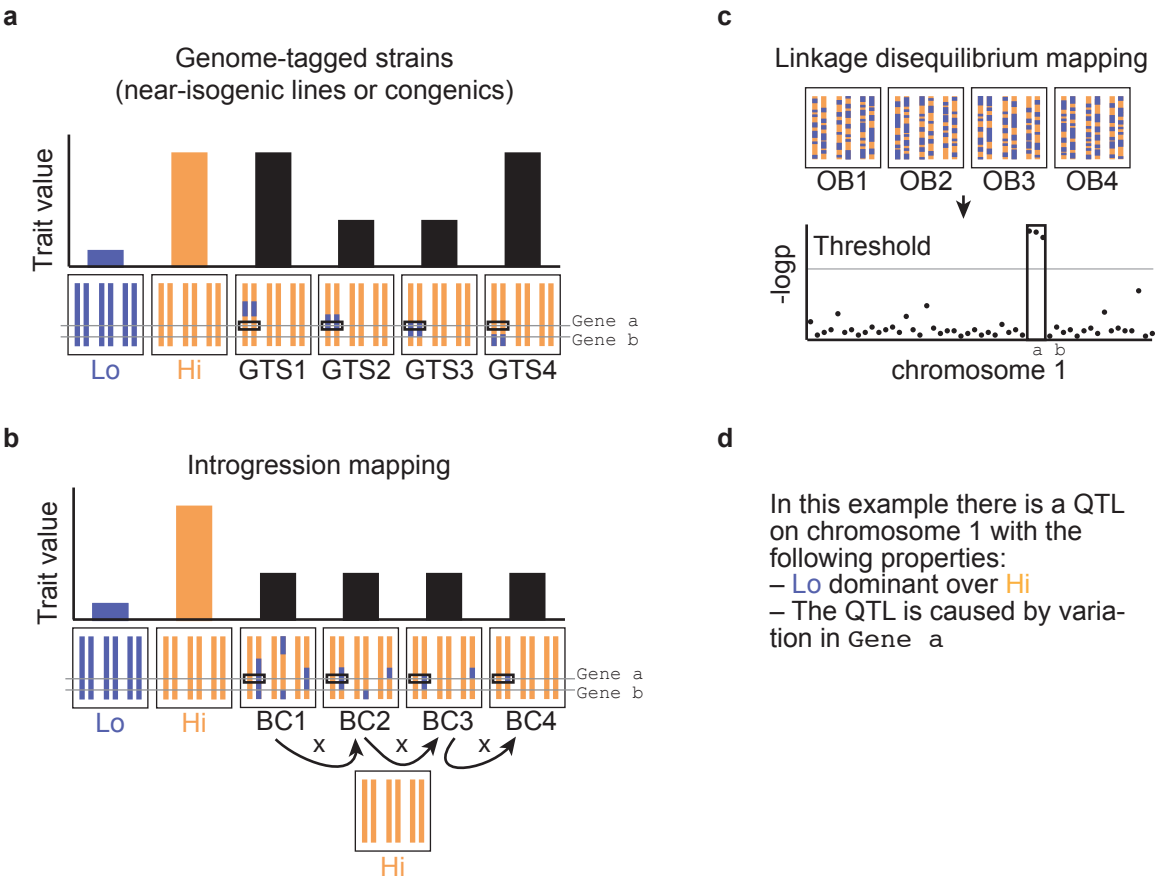

Figure 1.2

Fine-mapping of quantitative trait loci. Identified QTL can be narrowed down to smaller intervals by multiple mapping methods. **a)** Genome-tagged strains (GTS) with introgressed regions covering a QTL can be used to identify the region of overlap of introgressed DNA that contains the QTL activity. **b)** If pre-existing GTS between the relevant strains are unavailable, QTL can be fine-mapped by introgressing smaller portions of the QTL by successive backcrosses (BC), selecting for individuals that maintain the QTL activity and have progressively less DNA from the donor strain. **c)** QTL can also be fine-mapped by linkage disequilibrium mapping using outbred (genetically heterogeneous) individuals that have accumulated recombination events in the QTL over multiple generations. In this example, outbreds (OB) only have two haplotypes, but in reality outbred populations usually have more than two haplotypes. **d)** Genetic properties of the QTL in **a-c**. These methods identify the location of sequence variation that gives rise to trait variation but, strictly speaking, they do not identify the relevant genes, since sequence variation can affect genes located outside the QTL.

Linkage disequilibrium mapping. Another way to fine-map a QTL is by linkage disequilibrium association mapping in outbred populations, which relies on mapping strains that have accumulated multiple recombination events over many generations (**Figure 1.2c**). This approach was used to identify the mouse emotionality gene *Rgs2* (see main text). Linkage disequilibrium mapping is also the basis for genome-wide association studies done in humans, which map a QTL to a very narrow location.

Candidate genes. As an alternative to mapping, candidate genes of interest can be examined for natural variation. For example, natural alleles of the *Drosophila* circadian genes *period* and *timeless* are differentially distributed across tropical and temperate zones in wild flies, and affect temperature compensation and light regulation of the circadian clock [64,65]. Many other candidate behavioral genes that appeared promising in initial studies have failed to replicate, however, and the consensus in the field is that unbiased approaches are still necessary. One discovery-based approach to finding promising genes is genome-wide analysis of gene expression patterns, which can be applied to behaviorally-selected strains alone or in combination with QTL analysis (**Figure 1.3**). This strategy has been used to define molecular signatures and candidate genes associated with *Drosophila* geotaxis, locomotion, and aggression behaviors [66-69].

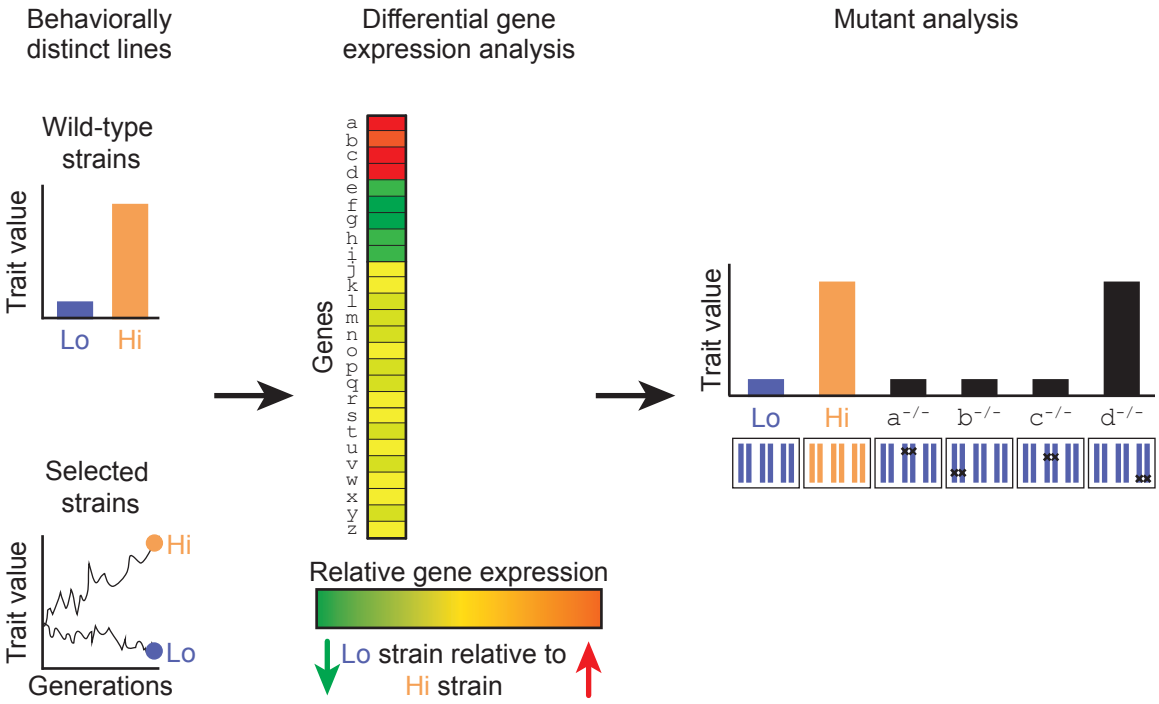

Figure 1.3

Differential gene expression analysis to identify genetic pathways mediating variation in behavior. Transcript abundance can be compared between two strains that differ in a behavior of interest. The role of genes that vary in expression between strains can be further explored by testing mutants in those genes for altered behavior. In this example, genes *a, b, c*, and *d*, are more abundant in the Lo strain relative to the Hi strain, but only loss of function mutations of *d* convert the behavior of the Lo strain in the direction of the Hi strain. This technique identifies genes with a biological role in behavior, but do not identify QTL *per se*.

Quantitative Trait Nucleotides (QTN), the polymorphisms responsible for a QTL, can affect transcriptional regulation or can lead to significant coding changes. A coding QTN affects the gene where the QTL maps, but QTN can affect transcription of distant genes. Some tests are better suited to confirm the relevance of QTN and others are more appropriate for identifying affected genes, or quantitative trait genes (QTG).

Quantitative complementation. The classical quantitative complementation analysis, first described in *Drosophila* [70] (explained in detail in [71]), tests the ability of null mutations to complement the QTL allele with reduced activity (usually the recessive QTL allele). In this test, strains carrying deletions or null mutations in genes within the QTL are crossed to both parental strains used for QTL mapping. An interaction between the QTL and the null mutation suggests that differential activity of the tested gene gives rise to behavioral variation. A null mutant can fail to complement because it is allelic to the QTG, or because a mutation in another gene interacts with the QTG. To minimize the effect of multigenic interactions, advanced quantitative complementation is best performed between strains with near-identical genetic backgrounds [61,44,63] (**Figure 1.4a**), for example, by introgressing both the QTL and the null mutation into a parental strain.

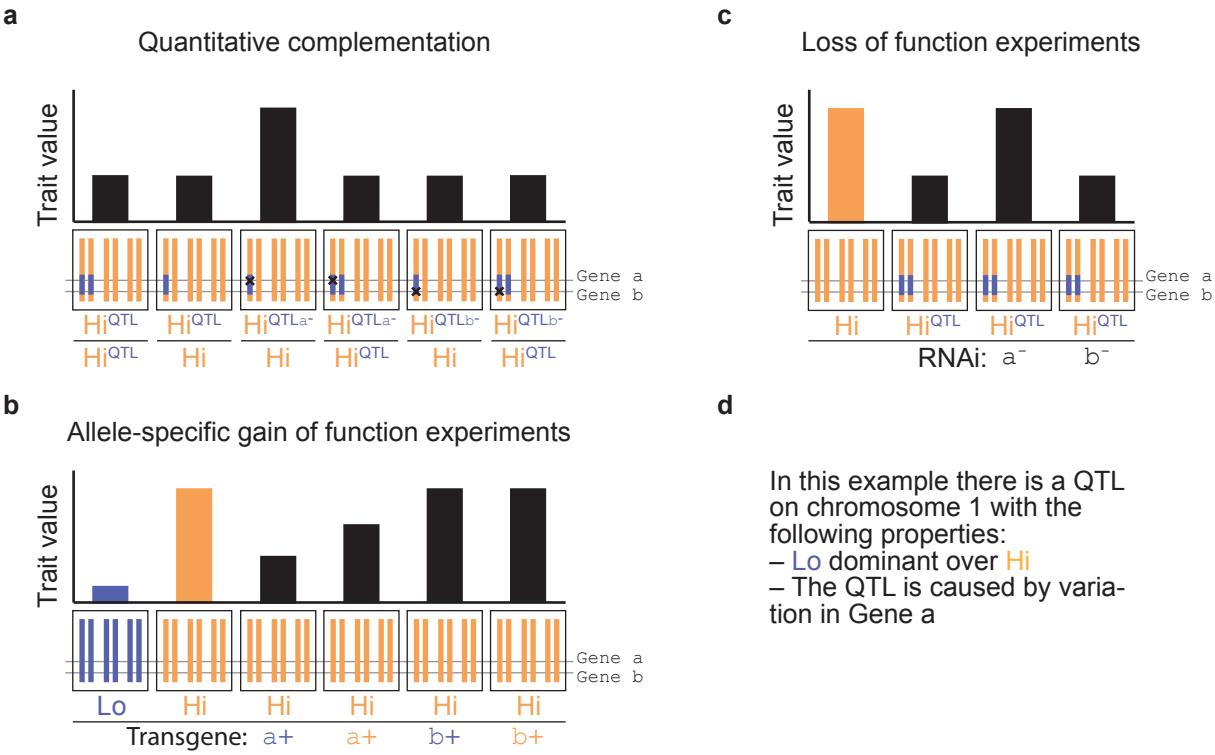

Figure 1.4

Functional identification of quantitative trait genes. QTL can be assigned to specific genes by genetic or molecular tests (**a-c**). **a)** Failure to complement in a quantitative complementation test suggests, but does not prove, that the QTL is allelic to the gene whose mutant is being tested. **b)** Gain of function experiments, such as transgenic overexpression of the QTG, can convert the strain with the recessive (low activity) QTL in the direction of the strain with the dominant (higher activity) QTL. Allele-specific gain of function can confirm that the dominant allele has higher biological activity than the recessive allele, further validation of the identification of a QTG. **c)** Knocking down the activity of QTG in the strain with the dominant (higher activity QTL) should convert the strain in the direction of the strain with the recessive (low activity) QTL. **d)** Genetic properties of the QTL in **a-c**. In this example, Hi and Lo strains refer to high and low phenotypic trait value, not to high and low QTL activity. Trait value and QTL activity do not have to be positively correlated. QTG from dominant QTL usually have comparatively higher biological activity than recessive QTL, but this is not always the case.

Gain- and loss-of-function transgenesis. Gain-of-function transgenesis can be performed on the strain carrying the recessive allele of the QTL, since the recessive allele normally has reduced activity compared to the dominant QTL [72] (**Figure 1.4b**). This approach is analogous to transgenic rescue experiments used to identify mutant alleles in forward genetic screens. Moreover, transgenic analysis can be done with DNA from both parental strains [61-63]; the expectation is that DNA derived from the strain with the dominant QTL is more potent at "rescuing" the behavior than DNA from the recessive strain, providing supporting evidence that the relevant QTN has been cloned. If DNA from both strains rescues equally, the QTG may have been cloned, but the QTN may not be present in the transgene.

Loss-of-function experiments in the dominant strain should provide the reciprocal answer to gain-of-function transgenesis in the recessive strain (**Figure 1.4c**). RNAi of QTG should usually transform the behavior of the dominant strain in the direction of the recessive strain [63].

From behavior to molecule, and back to behavior

Hundreds of QTLs that affect animal behavior have been detected in genetic crosses, but specific genes and gene variants for the QTL are just beginning to emerge from focused mapping approaches (described above. See **Figure 1.2**). An encouraging set of initial results suggests that behavioral genes

identified through these unbiased approaches can have conserved functions between invertebrates and vertebrates, including humans.

A cloned QTL for mouse emotionality. Out of 450 behavioral QTL that have been detected in mice [73], the first to be compellingly mapped to a specific gene was *Rgs2*, which emerged from the studies of emotionality described above [74]. The emotionality trait appeared genetically complex even in initial analysis, and fine-mapping led to the fragmentation of one strong QTL into three neighboring QTLs within the original region, a phenomenon that is commonly observed in quantitative genetics [75-78,44,63]. Along with fine mapping in outbred lines, the genetic proof that *Rgs2* was a relevant locus for emotionality came from quantitative complementation tests where the high and low emotionality naturally-occurring alleles were examined in heterozygous combination with an induced mouse knockout mutation in *Rgs2* [79].

The homozygous knockout mutation of *Rgs2* shows high anxiety, supporting the idea that this gene regulates emotionality. *Rgs2* encodes a regulator of G-protein signaling (RGS) that shortens the duration of G protein-coupled receptor (GPCR) signaling by stimulating GTP hydrolysis and inactivation of heterotrimeric G proteins [80]. The effect of the *Rgs2* knockout on anxiety-related behaviors suggests that *Rgs2* limits signaling of GPCR pathways that produce anxiety. The nature of the relevant GPCR might be understood by studying other phenotypes of *Rgs2* knockouts, which include hypertension with

evidence of increased sympathetic function and disruption of angiotensin and vasopressin GPCR signaling [81,82]. Both blood pressure and anxiety are strongly stress-responsive, suggesting that a common *Rgs2*-regulated system could limit stress responses in the brain and in peripheral tissues.

From animal QTLs to human behaviors? Emotionality in mice is a trait with encouraging similarities to human emotional traits such as anxiety. The brain regions involved in mouse emotionality include the amygdala, which is implicated in human fear-related behaviors [83,84]. Similarly, mouse emotionality has behavioral analogies to neuroticism, or emotional stability, in humans [85,74]. Polymorphisms in *Rgs2* have been tentatively associated with anxiety in humans [86], suggesting that emotionality and neuroticism may also have genetic similarities.

Another QTL approach to animal models of human behavior is to model features of human psychiatric disorders called endophenotypes, which are simpler markers correlated with the disorder [87]. Prepulse inhibition, the suppression of an acoustic startle response by a prior stimulus, is a behavior that is often diminished in schizophrenic patients [88], and it can be studied in animals to model some aspects of schizophrenia [89]. A QTL cross between two mouse strains identified six QTL that affect prepulse inhibition, as well as a number of loci associated with auditory sensitivity and other general behavioral traits [90]. One of the prepulse inhibition QTL is linked to Fatty acid binding protein 7

(Fabp7), and a Fabp7 gene-targeted mouse recapitulates the behavioral effect of the QTL. Although more needs to be done to strengthen the connection, the Fabp7-targeted mouse has reduced neurogenesis in the hippocampus, a developmental defect potentially consistent with neurodevelopmental defects of schizophrenic patients.

Endophenotypes are also useful for generating animal models of drug addiction and related behaviors. In humans, acute tolerance to the intoxicating effects of alcohol partly predicts alcohol addiction, and acute tolerance is easily modeled in animals. Many QTL for alcohol tolerance, dependence, and withdrawal have been mapped in rodents, including one tolerance QTL in rats tentatively assigned to the neuropeptide Y gene [91] and a withdrawal QTL in mice that probably corresponds to the multiple PDZ (MPDZ) gene [92].

Common genetic targets generate behavioral diversity in different animals. Some behavioral genes are conserved even between vertebrates and invertebrates: single-gene variants in the *period* gene can affect the circadian behavior of species as diverse as humans and flies [93,94,3,95]. Moreover, the repeated association of *period* with circadian and other behavioral variation both within and between species [96,64,94,3,95] suggests that some genes may be "hotspots" that are particularly likely to generate behavioral variation.

A striking example of a natural genetic hotspot crystallized from studies of *Drosophila* foraging behavior. *Drosophila* larvae fall into two groups based on

their foraging strategy, rovers (about 70% of the population) and sitters. Rovers travel greater distances in the presence of food than sitters, disperse more readily between different food patches, and pupate further from the food supply [97,98]. *Drosophila* larval foraging was the first naturally-varying behavior to be mapped to a specific gene of major effect, *foraging* (*for*) [99,72], which encodes the conserved cGMP-regulated protein kinase G (PKG). Rovers have more *for* mRNA in their brain than sitters, and higher PKG activity [72], suggesting that the rover allele is a high-activity allele. Among the targets of PKG regulation are ion channels that regulate neuronal excitability [100]. In an intriguing coincidence, mammalian PKG phosphorylates and regulates the mouse emotionality protein *Rgs2* [101].

The discovery of *for* in flies paved the way to an understanding of foraging behaviors in other invertebrates. Young adult honeybees engage in nursing activities in the hive, and older honeybees become foragers that leave the hive to retrieve food. Transcript levels and activity of *Amfor,* the bee orthologue of *for*, are higher in foragers than in nurses, and stimulation of PKG activity with cGMP analogues can induce premature foraging behaviors in young bees [102]. These results suggest that developmental regulation of PKG in an individual honeybee modifies its behavior. Another insect species where different behavioral forms vary in PKG activity is the red harvester ant, where the *for* orthologue is expressed at lower levels in foragers than nest workers (the opposite pattern from honeybees) [103]. An effect of PKG on behavioral variation extends to a

nematode worm, *Pristionchus pacificus,* that is attracted to its insect hosts by their pheromones. Natural variation in *Pristionchus* attraction maps to *Ppa-egl-4,* a homologue of the *for* gene [104]. The combination of genetic mapping and candidate gene analysis shows that modulation of PKG activity is a common mechanism for altering animal behavior: genetic variation in PKG distinguishes different flies and nematodes, and stage- or caste-specific PKG modulation affects behavior in individual honeybees and ants.

Genes and the environment: a principle in genetic variation

Many behaviors are triggered by sensory cues, and most are regulated by environmental context. The dichotomy between genetic (nature) and environmental (nurture) regulation of behavior is false: many genes that affect behavior do so by affecting an animal's *detection, response,* or *interaction* with environmental cues. Examples of each category appear below.

Sensory genes and the environment. Animals interact with the environment through different sensory modalities, and modifications of these systems appear to be a site of frequent behavioral adaptations.

Receptors for smell and taste belong to large gene families, and they represent the fastest-evolving neuronal genes in animal genomes, including the human genome (**Figure 1.5**). Modification of smell and taste receptors can lead to rapid changes in behavior, as can be illustrated by artificially-selected traits in

laboratory strains of the nematode *C. elegans*. High-density growth of *C. elegans* in the laboratory resulted in the deletion of two different pheromone receptor genes that regulate development based on population density [105]. Both pheromone receptor genes were deleted independently in two strains grown at high density in different locations. Moreover, a similar pheromone receptor gene was deleted following high-density growth of a different nematode species, *C. briggsae* [105]. Thus a shift in the environment (in this case, an artificial shift in density) can cause a reliable change in the repertoire of chemoreceptor genes. Another chemosensory gene of *C. elegans, glb-5,* has mutated in association with growth in a high-oxygen laboratory environment [62]. Reduced *glb-5* activity in the laboratory strain decreases the animal's sensitivity to oxygen [62,106] and affects other oxygen-regulated behaviors, such as their tendency to aggregate with other animals [62].

Changes in human chemoreceptor genes are also associated with specific sensory changes. Human polymorphism at the bitter receptor *TAS2R* leads to differences in perception of the bitter substance PTC [107], and polymorphism at the olfactory receptor *OR7D4* leads to differences in perception of androstenone odors [108]. This variation in sensory perception alters the ingestion of bitter food [109] and modifies physiological responses to odorants in human sweat, respectively [108,110].

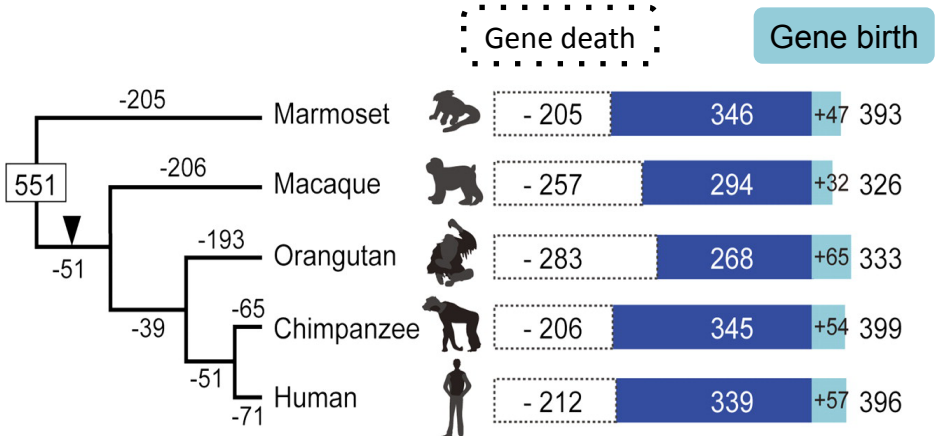

Figure 1.5

Olfactory receptor gene evolution in primates. Changes in the olfactory receptor (OR) gene repertoire in five primate species. Left, based on cross-genomic comparisons, the common ancestor of primates had at least 551 OR genes. Many OR genes have been lost along the five branches to modern primates (numbers of gene losses are shown on each branch). Right, the number of functional OR genes in modern primate species. Humans have lost 212 of the original 551 OR genes (white), retained 339 OR genes (dark blue), and gained 57 OR genes through gene duplication and divergence (light blue), for a total of 396 genes. The rate of change in human OR genes is similar to the rate in other primate lineages. Adapted from [111].

Odors, tastes, and pheromones reflect features of an organism's environment that change with diet, habitat, and population structure. Because there are so many chemoreceptor genes, each tuned to different chemicals, genetic changes to this set of molecules provide a simple path to modifying specific behaviors without deleterious effects. Variation in chemoreceptor genes between species that occupy different environmental niches carries this principle to the next evolutionary level. Well-defined examples include shifts in the olfactory receptor repertoire of specialist *Drosophila sechellia* fruit flies that feed exclusively on the noni fruit [112], and the loss of sweet taste receptors in carnivorous cats [113].

Visual systems also show evidence of rapid adaptation within and between animal species. Changes in the sequence and number of the visual opsin genes, which encode cone photoreceptor proteins, have occurred repeatedly in vertebrate evolution [114]. Humans and old world monkeys have a recent opsin gene duplication that allows red-green color discrimination (reviewed in [115]). New world monkeys lack this duplication, but show evidence of intraspecies variation: two alleles of a long-to-middle wavelength-sensitive (L-M) opsin gene on the X chromosome are maintained by balancing selection, and females that are heterozygous at this locus are able to discriminate more colors than hemizygous males or homozygous females [116].

At a higher level, sensory systems can rapidly remodel the design or number of sensory organs to change behavior. For example, the tetra fish

Astyanax mexicanus exists in two forms, one sighted and surface-dwelling, the other blind and cave-dwelling. The loss of sight in cave-dwelling populations has been accompanied by expanded cell numbers in the mechanosensory organs of the lateral line, an adaptation that increases sensitivity to vibrations from food falling on the water surface [117]. In humans, genes required in the auditory system show signatures of positive selection that may be related to a sophisticated human ability, the use of language [118].

Genes and the response to environmental cues. An animal's sensitivity to environmental cues at any given moment is determined not only by its sensory receptors, but also by shifting internal states. For example, hungry animals are more sensitive to attractive food-related cues, and less sensitive to aversive cues, than they are when well-fed. This example describes variability within one individual, but the interface between internal and external cues also represents a site for behavioral variation between individuals. An example of natural variation at this interface is an animal's choice whether or not to abandon a depleting food supply, which is known in behavioral ecology as the exploration-exploitation decision [119]. In *C. elegans,* as in other animals, abandoning a food supply is strongly modulated by environmental cues, including food quality, food quantity, and animal density [120,121]. It is also modulated by genetic variation, and has been studied using recombinant inbred lines from two *C. elegans* strains [43] that differ in their tendency to leave food (this is the subject of Chapter 2 of this Thesis; see

also [63]). One QTL for the exploration-exploitation behavior corresponds to the G-protein coupled Tyramine Receptor-3 (TYRA-3), which is related to vertebrate adrenergic receptors. Noncoding polymorphisms in *tyra-3* alter its expression levels in sensory neurons that detect food cues [63], and apparently modulate sensitivity to those food cues. The effect of *tyra-3* is only observed at intermediate food levels, whereas all animals, regardless of the *tyra-3* allele, remain on abundant food and abandon low amounts of food [63]. These results show how genetic variation interacts with the environment to regulate behavior: internal arousal states, signaled through adrenergic receptors, can couple strongly or weakly (depending on the adrenergic receptor allele) with a sensory input that modulates behavior.

The ligand for *tyra-3* is tyramine [122], one of several invertebrate neurotransmitters related to vertebrate adrenaline and noradrenaline [123]. This class of transmitters is linked to arousal states in invertebrates and vertebrates [124]. Exploration versus exploitation decisions in primates are regulated by noradrenaline release [125], suggesting that analogous molecular pathways might have ancient roles in decision-making.

Genetic variation and the environment act on common substrates. Another connection between genetic and environmental regulation of behavior is illustrated by studies of *Drosophila* aggression. Fruit flies defend food resources or potential mates with attack behaviors like lunging and boxing [126]. The genetic

underpinnings of these behaviors have been analyzed by QTL approaches [44] and by selective breeding strategies in which the most aggressive flies in genetically heterogeneous populations were selectively mated for more than 20 generations [67,127]. Both approaches found multigenic effects on aggression.

As a way to identify candidate loci involved in aggression, microarray analysis was used to search for genes that were differentially expressed between highly aggressive and less aggressive flies derived from selective breeding or from random inbreeding of wild-derived populations [67,127,128] (see **Figure 1.3**). The overall transcriptional differences were substantial in three separate studies, but the gene number and specific gene sets identified were largely non-overlapping. A possible explanation for this finding is that outbred populations are so diverse that multiple independent combinations of alleles can lead to highly aggressive behavior or non-aggressive behavior. Nonetheless, 25 of the differentially expressed genes had effects on aggression when tested using knockout alleles, supporting the validity of the candidate transcripts as regulators of behavior [67,127,128]. The genes that are differentially expressed in microarrays may be functional QTLs, or they may be indirect targets of the underlying genetic processes; linkage or identification of a mutation would be necessary to show that natural variation segregates at these loci.

One interesting and informative gene that emerged from the artificially selected aggressive lines was *Cyp6a20,* which encodes one of multiple cytochrome P450 enzymes in *Drosophila*. Expression of this gene is low in

aggressive strains compared to controls, and reduced expression is sufficient for behavioral differences in aggression [67]. *Cyp6a20* is expressed in olfactory sensory organs, where it may regulate the responses to pheromones that influence aggression [129]. Interestingly, mRNA levels of *Cyp6a20* are reduced in flies that are reared in isolation, which are more aggressive than socially experienced flies [129]. This observation suggests that social experience modulates aggression by changing *Cyp6a20* levels. Thus *Cyp6a20* sits at the intersection of genetic and environmental influences on behavior: either a genetic change or an environmental change that decreases *Cyp6a20* expression leads to increased aggression.

Gene-environment interactions are strongly supported in humans as well, and may form a framework for understanding many psychiatric disorders and risk factors. In depression, for example, genetic susceptibility (having an identical twin who is depressed) interacts with environmental insults, like divorce or death in the family, to give a superadditive effect [130].

The next step: from genes to circuits that affect behavior

A genetic change that affects behavior acts in the context of the neural processes that generate and regulate the behavior. In some cases, such as changes in sensory receptor genes, this relationship is straightforward. In other cases, studying the behavioral gene can provide new insights into brain circuits, as is illustrated by two examples from studying genes that affect social behavior.

***From a social gene to a social circuit in* C. elegans.** Most *C. elegans* strains are social feeders that aggregate in the presence of food, but the laboratory strain N2 is a solitary feeder. Its low levels of aggregation are associated with a strong-effect, high-activity allele of a neuropeptide Y receptor homolog, *npr-1,* that differs from a low-activity (high aggregation) allele at a single amino acid residue [61]. Both alleles were originally thought to occur in the wild, but closer examination revealed that the high-activity solitary allele arose during laboratory cultivation [62] and increases fitness in the laboratory environment [131,132].

An advantage to studying behavioral variation in *C. elegans* is the ability to examine the neural circuits regulated by a genetic variant. *npr-1* is expressed in ~10% of *C. elegans* neurons, but careful mapping of its site of action showed that its influence on aggregation is dominated by its effects on a single pair of integrating neurons called RMG neurons [133]. RMG neurons are essential for aggregation, and are linked by gap junctions to multiple classes of sensory neurons that detect oxygen, pheromones, noxious cues, and nutrients. Aggregation is associated with high levels of all of these sensory cues, suggesting that RMG couples multiple sensory inputs to drive a common behavior. The high-activity laboratory *npr-1* variant partially uncouples this circuit to diminish aggregation behavior without disrupting other important roles of the sensory cues. The discovery of this circuit element via the *npr-1* variant shows how genetic approaches can advance neurobiological studies.

Differential gene expression pattern leads to changes in behavior: the case of neuropeptide receptors. Variation in social behaviors is commonly observed within and between mammalian species, and here too genetic studies have provided fresh insights into the neurobiology of social behavior. Two related neuropeptides, oxytocin and arginine-vasopressin (AVP), are important regulators of mammalian social and reproductive behavior [134]. Genetic variation in AVP signaling has been linked to rodent social behavior through cross-species comparisons of monogamous prairie voles and polygamous montane voles [135]. AVP is released during mating, and promotes male pair-bonding and paternal behavior when injected into ventricles of the monogamous male vole, but does not have this effect on the polygamous male vole [136]. Conversely, antagonists of AVP block male pair-bonding in monogamous voles [137]. Both vole species have functional AVP genes and functional vasopressin receptor genes, but they differ in their expression of the vasopressin 1a receptor (V1aR). A brain region involved in the neurobiology of reward called the ventral pallidum only expresses V1aR in monogamous voles [138] and, remarkably, affiliative behavior of polygamous montane voles is substantially increased by virally-mediated introduction of V1aR into the ventral pallidum [139]. These results implicate differential expression of the V1aR neuropeptide receptor in the differential organization of social behaviors in the two vole species. They also point to the ventral pallidum as a site that can encode rewarding features of social cues.

Social behaviors are central to human experience, and their disruption is a key feature of human autism and schizophrenia. Little is known about the human circuits for social behavior, but the rodent pathways provide a starting point for further investigation.

Emerging themes in the genetics of behavior

Is it possible to derive general principles about natural variation and the evolution of behavior, by analogy with common principles that have been uncovered in evolutionary developmental biology? Natural variation in the PKG gene affects foraging behavior in both insects and nematodes, and variation in the *period* gene affects circadian rhythm in flies and humans, a promising start. Other indications of common themes are not so strongly tied to a single gene, but may be tied to classes of genes, like the sensory receptor genes described above. We suggest that highly evolvable behavioral genes will be characterized by *diversity,* exemplified by multigene families, and by *modular flexibility*, the ability to form new behavioral connections easily.

Adaptable neuromodulatory pathways. Several behavioral trait genes are associated with G-protein coupled neurotransmitter receptors or their regulators: mouse *Rgs2,* vole *avpr1a*, and nematode *tyra-3* and *npr-1*. In each case, the GPCR system is associated with internal motivational states – anxiety, affiliation,

arousal, or hunger – that set thresholds for behavioral responses to external stimuli.

The amenability of GPCR pathways to natural variation matches their diversity and modular flexibility. All animal genomes encode dozens of GPCRs for neuropeptides and for modulatory bioamines that modify neuronal excitability and synaptic strength. These modulators are typically not essential for core neurotransmission and this, coupled with their variety, leaves room for evolvability. Moreover, neuromodulators can act at a distance and not just at local synapses, which allows them to broadcast internal motivational or arousal states. Action at a distance enables the creation of new behavioral links between distant brain areas simply by modifying the site of receptor expression, without requiring growth of new anatomical connections. In agreement with the hypothesis that neuromodulators are substrates for behavioral diversity, neuropeptides and neuropeptide receptor expression patterns evolve rapidly. Expression of oxytocin and vasopressin receptors is highly variable in different rodents [140,141], and cross-species comparisons of the stomatogastric ganglion of crustaceans show a near-invariant set of neurons, but divergence in neuropeptide expression [142,143].

Genetic variation in GCPRs and other regulators of neurotransmission has been associated with human behavioral and psychiatric traits. For example, neurotransmitter transporters have been implicated in depression [144,145] and the DRD4 dopamine receptor is implicated in novelty seeking [146]. Recent studies

have discovered additional variants in GPCRs that may increase the risk of psychiatric disorders: rare microduplications in the vasoactive intestinal peptide receptor 1 (VIPR1) in schizophrenia [147], a polymorphism in the HTR2B serotonin receptor found in Finns with severe impulsivity [148], and common polymorphisms at the pituitary adenylate cyclase-activating polypeptide (PACAP) receptor PAC1 in post-traumatic stress disorder [149]. A cautious stance to human association studies is warranted, since promising results have often failed to maintain significance upon meta-analysis [150,151]. These failures of replication, however, might reflect real genetic effects that are specific to a population or sensitive to genetic backgrounds.

Balancing selection for behavioral traits. Taking a step back from the specific genes that affect behavior, why does behavioral variation persist within a species? Genetic variation is generated by mutation and maintained through drift, population-specific selection, or balancing selection. Balancing selection maintains trait variation because either of two alleles can be advantageous under different circumstances: two alleles can be balanced if a heterozygote is more successful than either homozygote, if each of the two alleles is better-adapted to one of two alternative environments, or if each allele promotes a different, but equally successful survival strategy in the same environment [152,153]. Emotionality traits in mice are potentially subject to balancing selection in different environments: a predator-rich environment may favor animals that are

highly responsive to potentially dangerous stimuli, whereas a predator-poor environment may relax that selective pressure and favor bolder animals [154]. Foraging activity is another behavioral axis subject to balancing selection: high activity levels that promote exploration of resource-poor environments may be more or less advantageous than low activity levels that conserve energy resources, depending on the environment. The rover and sitter alleles of *Drosophila* larvae are maintained in wild populations by balancing selection of an interesting kind: different alleles are favored depending on the density of larvae and the relative frequency of rover and sitter alleles in the population [155,156]. Density-dependent and frequency-dependent selection are special cases of balancing selection that are relevant to social behaviors as well as foraging.

In this analysis, the reproducibility of an animal's environment should have predictable effects on its genetic variability. If an important environmental cue is entirely reliable, like the circadian cycle in the tropics, information about that cue should be reliably encoded by the genome. If an environmental cue is entirely unreliable, animals may learn about it from individual experience. In the intermediate domain, information about food supply, predators, weather patterns, or population density may be variable or constant, and balancing selection may encode different degrees of genetic versus experience-dependent behavior, accordingly.

Human populations may also be subject to balancing selection for behavioral adaptations. In that context, it may be fruitful to consider traits that

are frequently under balancing selection in animals, such as foraging strategies, activity levels, and sensitivity to threat.

Perspective

The analysis of natural variation in behavior has convincingly shown a complex genetic basis and pervasive interactions between genetic variants and the environment. Current excitement focuses on identifying more of the molecules involved in behavioral variation, and translating these genetic discoveries into neurobiological and evolutionary insight.

Thesis overview

The topic of Chapter 2 of my thesis is natural variation in exploratory behavior in *C. elegans*, manifested by the decision to abandon a depleting food patch. I will describe the identification of polymorphisms in the monoamine receptor *tyra-3* and how these polymorphisms modify the neuronal circuits that participate in the decision to abandon food.

On Chapter 3 I describe the identification of natural variation in the GABA receptor *exp-1* and how it contributes to variation in the social behavior of *C. elegans* wild-type strains.

A general analysis of my thesis and ideas for future experiments are discussed in Chapter 4.

"There's a feeling I get when I look to the west and my spirit is crying for leaving"

–Robert Plant

CHAPTER 2: Tyramine receptor polymorphisms affect exploratory behavior in *C. elegans*

Summary

Innate behaviors are flexible: they change rapidly in response to transient environmental conditions, and are modified slowly by changes in the genome. A classical flexible behavior is the exploration-exploitation decision, which describes the time at which foraging animals choose to abandon a depleting food supply. Here I use quantitative genetic analysis to examine the decision to leave a food patch in *Caenorhabditis elegans*. I found that patch-leaving is a multigenic trait regulated in part by naturally-occurring noncoding polymorphisms in *tyra-3,* which encodes a G protein-coupled tyramine receptor related to vertebrate adrenergic receptors. *tyra-3* acts in sensory neurons that detect food-related cues, suggesting that the internal monoamines detected by *tyra-3* regulate responses to external conditions. These results indicate that genetic variation and environmental cues can converge on common circuits to regulate behavior, and suggest that monoamines have an ancient role in regulating behavioral decisions.

Introduction

Despite abundant evidence for heritability of behavioral traits within and between species, only a few naturally varying traits have been associated with polymorphisms in specific genes [157]. Foraging for food is an ecologically relevant, environmentally regulated behavior that is suitable for genetic analysis, as it can differ between populations of a species that live in different habitats [158]. An essential foraging decision is the choice between exploiting existing resources and exploring other options that may provide new resources. This decision can be described by Charnov's marginal value theorem, which proposes that the optimal time for an animal to leave a foraging ground occurs when local resource levels fall below the average level in the entire habitat [159]. The marginal value theorem was developed for animals foraging for food in patchy environments, but has analogies with diverse decision-making processes in field biology, cognitive neuroscience, and economics [160,161,125,158]. The theoretical and predictive successes of the marginal value theorem raise a series of mechanistic questions. First, to what extent is the decision innate and genetically encoded, and to what extent is it learned through individual experience? Second, how does the nervous system enact the comparison between current conditions and the decision threshold, and transform it into an adaptive behavior?

Studies of patch-leaving behavior in the nematode *C. elegans* have revealed innate, environmental, and experience-dependent factors that affect its foraging decisions. *C. elegans* rarely leaves a dense lawn of high-quality

bacterial food [121,162], but more frequently leaves lawns of pathogenic bacteria or lawns that are spiked with chemical repellents [163,164]. An animal's experience with high-quality food makes it more likely to leave a low-quality lawn [121]. Males will leave lawns that do not contain potential mates [165], while hermaphrodites leave lawns when animal density is high [166]. In addition, wild-type strains vary in their propensity to leave bacterial lawns based on a genetic polymorphism that affects the G protein-coupled neuropeptide receptor NPR-1 [166,167,131]. The standard laboratory strain N2, which has a high-activity allele of *npr-1* [61] remains on lawns of high-quality food and leaves lawns of pathogenic bacteria, whereas animals with a low-activity allele of *npr-1* that differs at one amino acid are less likely to leave a pathogenic lawn and more likely to leave high-quality food [166,167,131]. This *npr-1* polymorphism affects many foraging behaviors; low-activity *npr-1* strains aggregate into social feeding groups, move quickly on food, and have altered responses to oxygen, carbon dioxide, and pheromones compared to the N2 laboratory strain [61,168-170,133,62]. The high-activity allele of *npr-1* in N2 arose in the laboratory, probably as an adaptation to laboratory conditions [62], so it is not known whether genetic variation affects *C. elegans* foraging in natural environments.

Natural genetic variation within a species can generate diversity in foraging behavior, as exemplified by the polymorphic *Drosophila melanogaster foraging* (*for)* gene, which encodes a cGMP-dependent protein kinase [72]. A low-activity allele of *for* is present in *Drosophila* sitter larvae, which move slowly on a

food patch; a high-activity allele of *for* is present in rover larvae, which move quickly and disperse rapidly [171]. A *for*-related cGMP-dependent kinase affects foraging in honeybees, ants, and nematodes, suggesting that diverse animals share molecular mechanisms for behavioral regulation [104,171].

To gain further insight into the genetics and neurobiology of lawn-leaving behavior in *C. elegans*, I used quantitative genetic analysis to examine its genetic architecture in wild-type strains, and show that genetic variation in multiple loci, including a tyramine receptor, interacts with environmental conditions to regulate the exploitation-exploration decision.

Multiple loci affect leaving behavior

Different wild-type strains of *C. elegans* vary in their tendency to leave or remain on a standardized small lawn of bacterial food (**Figure 2.1a**). For example, adult hermaphrodites from the laboratory strain N2 leave the lawn only once every 100 minutes, whereas animals from the CB4856 (HW) strain isolated from pineapple fields in Hawaii leave the lawn once every 5-6 minutes (**Figure 2.1b**). To determine the genetic architecture of this behavioral difference between N2 and HW, I quantified leaving rates in 91 N2-HW recombinant inbred advanced intercross lines (RIAILs) [43]. 58 of the RIAILs had low leaving rates comparable to N2, only 6-10 had high leaving rates comparable to HW, and 23 had intermediate rates (**Figure 2.1c**). The excess of low leaving rates and the continuous

behavioral distribution in RIAILs suggest that leaving is a multigenic quantitative trait.

Quantitative trait locus (QTL) analysis across 1454 informative single nucleotide polymorphisms (SNPs) of the RIAILs uncovered two regions with significant effects on leaving rates, one on the X chromosome and one on chromosome II (**Figure 2.1d**). The autosomal QTL are covered in Appendix 2. The X chromosome QTL overlapped with the location of the polymorphic G protein-coupled neuropeptide receptor NPR-1, which affects many food-related behaviors [61,166]. The *npr-1* polymorphism has previously been shown to affect leaving [166], as well as locomotion speed on food [61], a behavior that partially correlates with leaving rate (**Figure 2.2**). Examining the *npr-1* genotype in the RIAILs revealed a strong but asymmetric correlation with leaving rates (**Figure 2.1c**). Every strain with the N2 allele of *npr-1* had low leaving rates (≤1 event every 20 minutes), but strains with the HW allele of *npr-1* could have either low or high leaving rates (**Figure 2.1c**). The asymmetric distribution is consistent with a role for *npr-1* in leaving behavior, but indicates that *npr-1* has epistatic interactions with other loci segregating in the RIAILs.

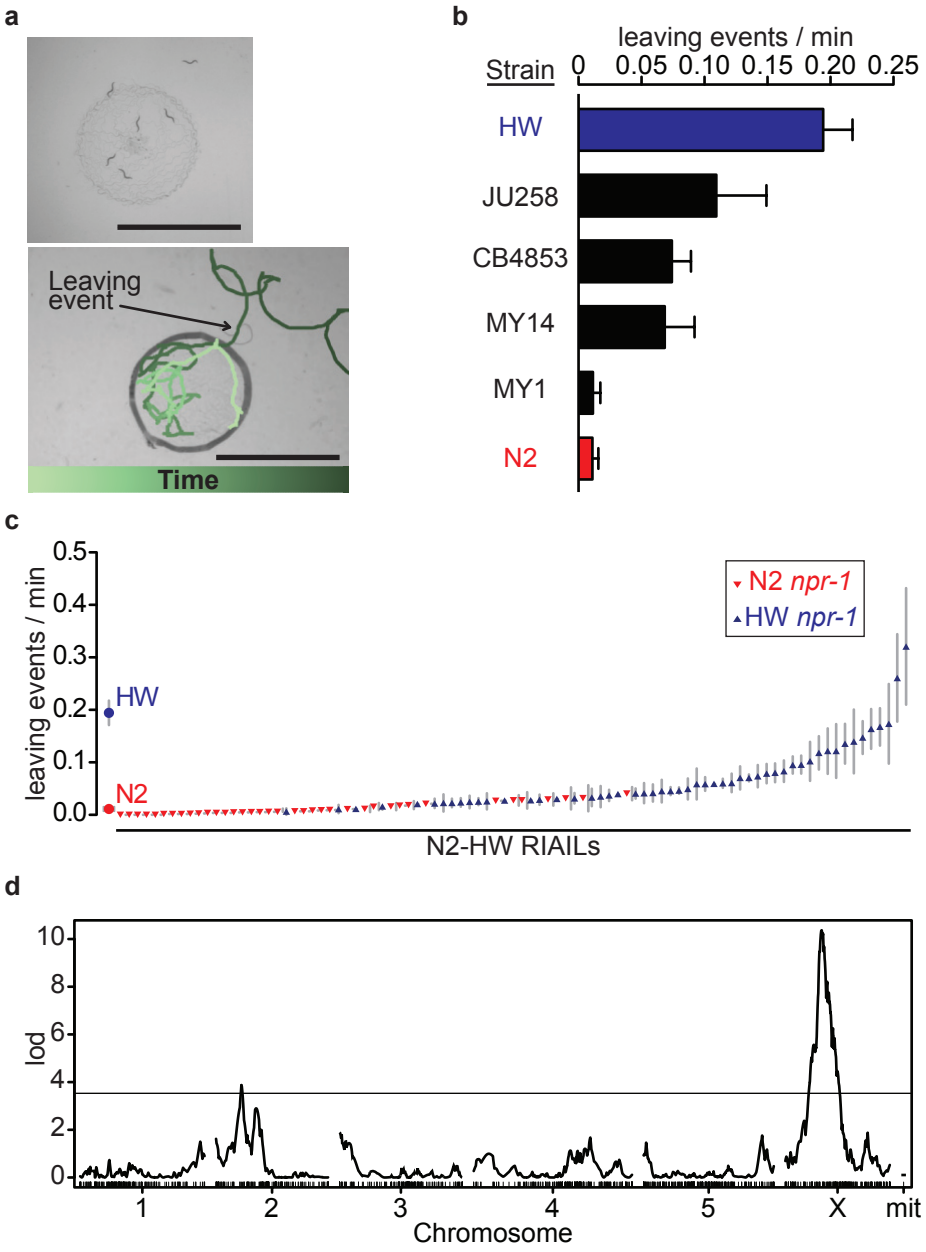

Figure 2.1.

Lawn-leaving behavior varies between wild-type *C. elegans* strains.

a) Lawn-leaving assays. Top: Six adult HW hermaphrodites on a bacterial lawn. One animal has left the lawn and one is leaving. Bottom: Track of a HW animal during 5 min of an assay; colour shows passage of time. The border of the lawn is outlined. Scale bar, 6 mm. **b)** Leaving rates of six wild-type strains. **c)** Leaving rates of 91 N2-HW recombinant inbred advanced intercross lines (RIAILs) [43] and parental strains. **d)** QTL analysis of RIAILs shown in **c**. The horizontal line denotes the $P < 0.01$ genome-wide significance threshold. Error bars indicate s.e.m.

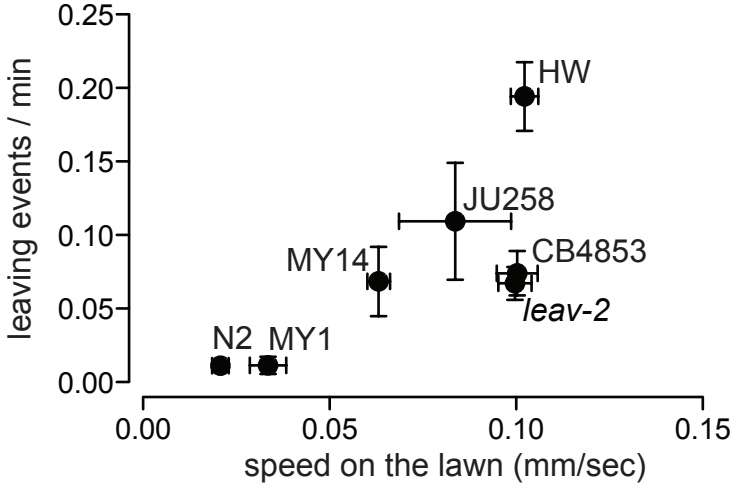

Figure 2.2

Locomotion speed on a food patch correlates with leaving rate. Correlation between locomotion speed on a small bacterial lawn and leaving rate in wild-type strains examined in Figure 2.1. The *leav-2* strain (Figure 2.4a), which has an N2 allele of *tyra-3* in HW background, is shown for comparison.

The involvement of *npr-1* in leaving behavior was confirmed by analyzing near-isogenic lines (NILs) containing the N2 and HW *npr-1* alleles in the reciprocal strain background, and by examining *npr-1* null mutants (**Figure 2.3**). Specific transgenic expression of the N2 *npr-1* allele in its essential site of action, the RMG motor neurons [133], sharply reduced the leaving rate of HW animals (**Figure 2.3**). Thus *npr-1* is a regulator of HW leaving rates, but not the only contributing gene.

tyra-3 affects leaving behavior

Studies in yeast, flies, mice, and plants have shown that individual QTLs often resolve into several genes that contribute to phenotypic variance [76-78,44]. Similarly, fine-mapping of the ~ 1 Mb QTL that contained *npr-1* suggested the existence of multiple loci that affected leaving rates. A NIL with <150 kb of N2 DNA spanning the *npr-1* locus introgressed into HW had N2-like leaving rates (*leav-1* QTL, **Figure 2.3** and **Figure 2.4a**). A second NIL with 700 kb of N2 DNA that did not cover *npr-1* introgressed into HW also had a low leaving rate, with about half the leaving rate of HW (*leav-2* QTL, **Figure 2.4a**). These results suggest the existence of a second X-linked locus that affects leaving rates, which I called *leav-2*. The *leav-2* region did not affect leaving in the N2 genetic background (**Figure 2.4a**), so all subsequent experiments were conducted in the HW background.

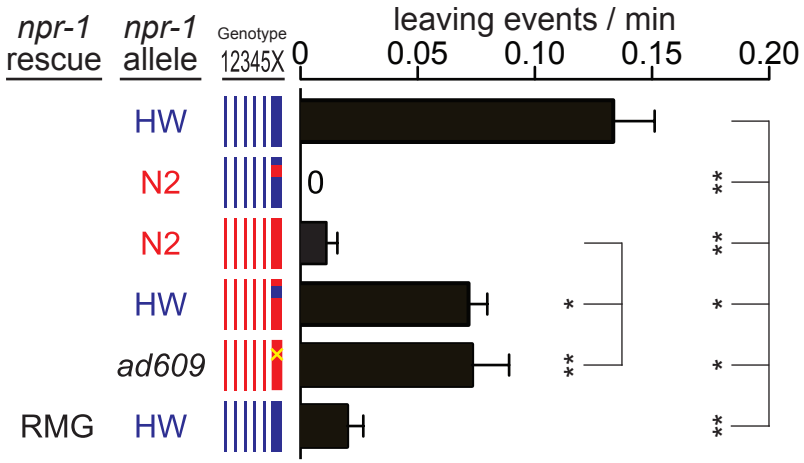

Figure. 2.3

***npr-1* affects leaving behavior.** Near-isogenic lines replace *npr-1* in each strain with the other strain's allele. The N2 allele of npr-1 has high activity compared to the HW allele; *npr-1(ad609)* is an EMS-induced loss-of-function allele of *npr-1*. Transgenic RMG expression of N2 *npr-1* in HW animals was achieved using an intersectional Cre-Lox strategy with two transgenes [133]. Error bars indicate s.e.m. * $P < 0.05$, ** $P < 0.01$ by ANOVA with Dunnett test.

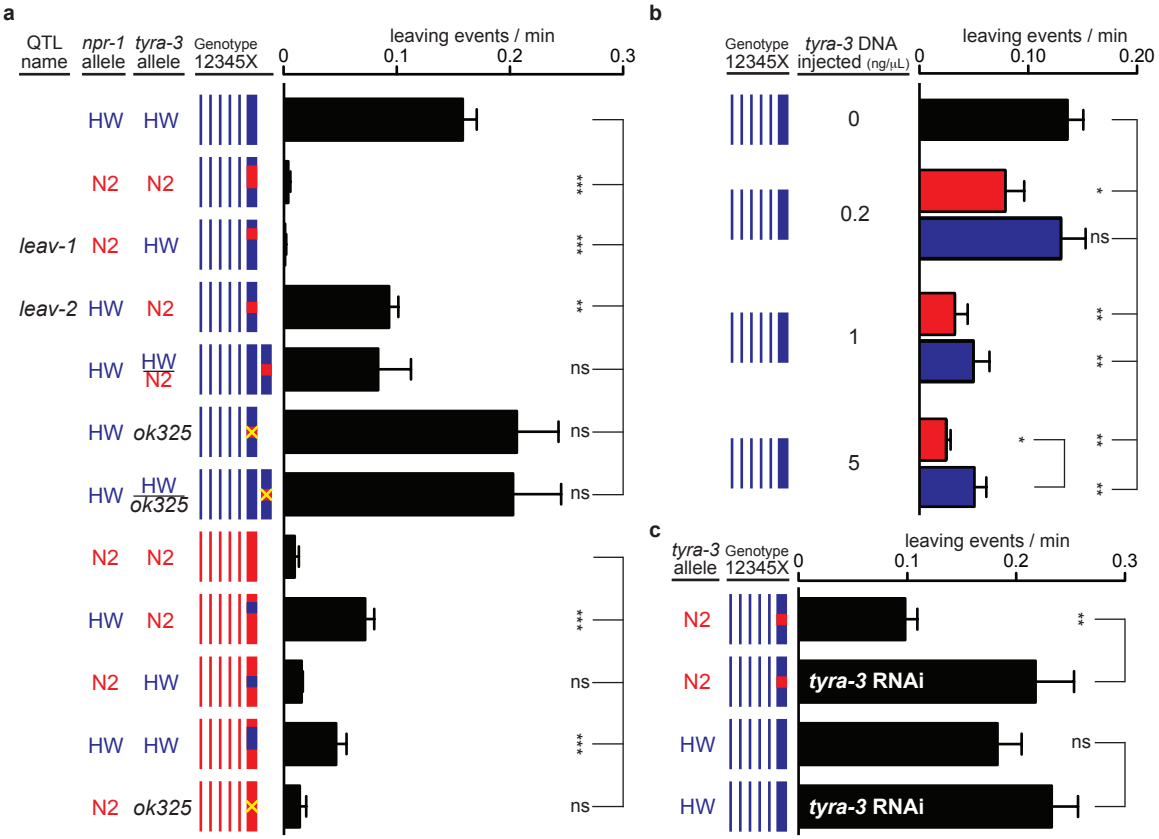

Figure 2.4

N2 and HW *tyra-3* alleles differentially affect leaving rates. **a)** Dissection of the QTL on X into two loci: *leav-1* (4.70-4.78 Mb) and *leav-2* (4.78-5.75 Mb). 'Genotype' shows chromosomes; thick line is X chromosome. Blue denotes HW DNA, red denotes N2 DNA, and yellow denotes the *tyra-3(ok325)* null mutant. In heterozygous strains, both X chromosomes are diagrammed. **b)** *tyra-3* genomic fragments (Figure 2.8a) reduce HW leaving rates. Blue, HW transgenes; red, N2 transgenes. Two-way ANOVA showed significant effects of both transgene concentration and DNA strain of origin. **c)** Effect of *tyra-3* RNAi. Error bars indicate s.e.m. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ by t-test or ANOVA with Dunnett test.

A 100 kb minimal region for *leav-2* was identified by analyzing the breakpoints of individual RIAILs (**Figure 2.5** and Methods). I characterized the genetic properties of *leav-2* by crossing the *leav-2* NIL strain with HW. The heterozygous F1 progeny had leaving rates similar to the *leav-2* NIL (**Figure 2.4a**), indicating that the N2 *leav-2* locus was dominant to HW and suggesting that N2 transgenes covering the relevant gene should reduce the leaving rate of HW animals. Therefore, overlapping N2 genomic DNA fragments from the 100 kb minimal *leav-2* region were introduced into HW animals by microinjection (**Figure 2.4b** and **Figure 2.6**). A single gene in this region reduced leaving rates: *tyra-3,* which encodes a G protein-coupled receptor for the invertebrate norepinephrine-like neurotransmitters tyramine and octopamine [122]. Tyramine and octopamine receptors are related to vertebrate adrenergic receptors, and are thought to carry out analogous functions. *tyra-3* genomic fragments from the N2 strain were more active than *tyra-3* fragments from the HW strain injected at the same concentration, consistent with the possibility that *tyra-3* is a polymorphic gene that differs between N2 and HW (**Figure 2.4b**).

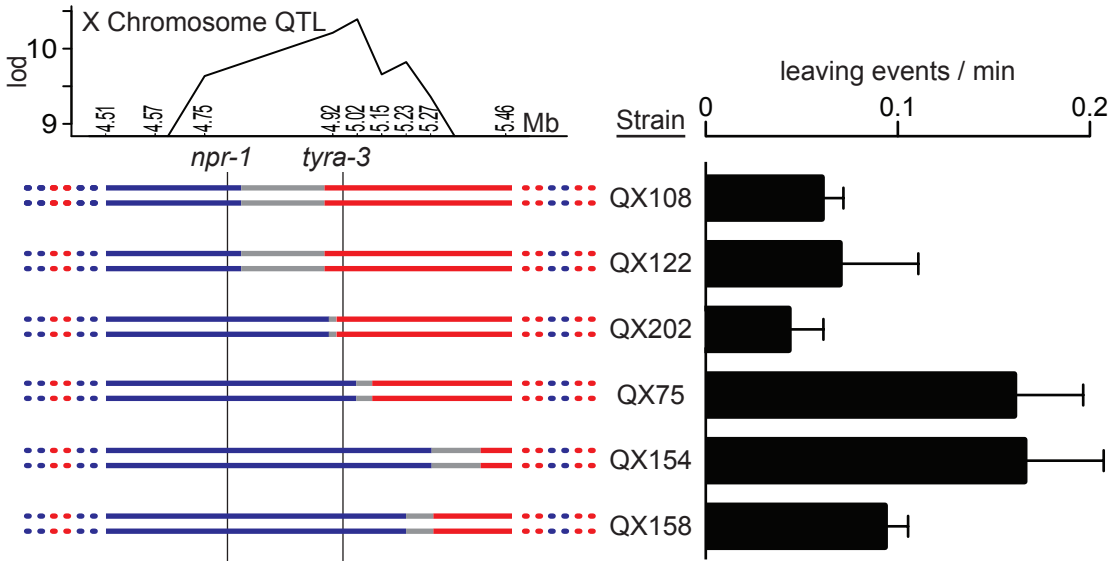

Figure 2.5

Phenotype-genotype correlations in RIAILs. Leaving behavior of recombinant inbred advanced intercross lines (RIAILs) with HW *npr-1* and a breakpoint to the left of 5.75 Mb; these six RIAILs were used to define a potential location for the QTL in *leav-2* strain. Blue denotes HW DNA, red denotes N2 DNA, and grey denotes breakpoints. QX108, QX122, and QX202 have lower leaving rates than QX75, QX154, and QX158. The QX158 strain appears to have a lower leaving rate than QX75 and QX154, but after backcrossing the X chromosome into the HW strain its leaving rates were indistinguishable from HW.

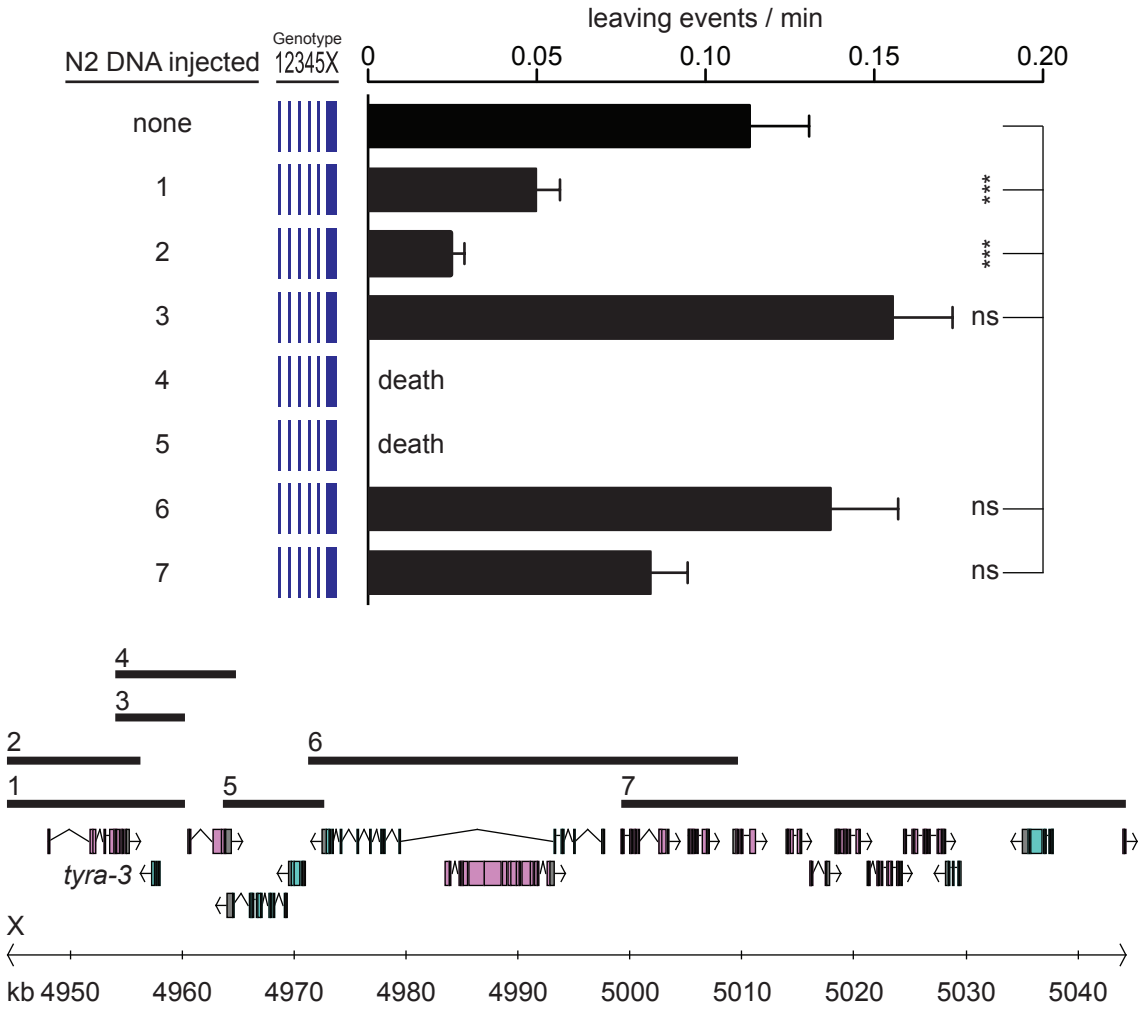

Figure 2.6

***tyra-3* is the gene affected by the *leav-2* QTL.** Leaving rates of transgenic HW animals injected with N2 DNA covering segments of the inferred position of the *leav-2* QTL. Three independent transgenic lines were tested for each segment; two segments caused lethality upon injection and could not be scored. Error bars indicate s.e.m. *** $P < 0.001$ by ANOVA with Dunnett test.

If *leav-2* corresponds to *tyra-3,* a *tyra-3* mutation should eliminate its activity [71]. To test this prediction genetically, a null allele of *tyra-3* in an N2 background was introgressed into a HW background. The N2 region in the resulting NIL covered from 4.9 to 5.4 MB of the X chromosome, the inferred position of *leav-2*. The *tyra-3(ok325)* null NIL had high (HW-like) leaving rates, suggesting that N2 *leav-2* activity was not present in the strain (**Figure 2.4a**). Heterozygotes between HW and the near-isogenic *tyra-3(ok325)* null strain also had high leaving rates (**Figure 2.4a**). These results are as expected if the active locus in *leav-2* is *tyra-3;* however, other genes within the introgressed regions could also contribute to the different leaving rates.

To strengthen the connection between *tyra-3* and *leav-2*, RNAi against *tyra-3* was performed in the *leav*-2 NIL that has low leaving rates due to the presence of the N2 QTL. Knockdown of *tyra-3* increased the leaving rate of the *leav-2* NIL to levels observed in HW animals, the result predicted if the *tyra-3* locus from N2 reduces leaving (**Figure 2.4c**). Comparable experiments in a pure HW strain had minimal effects, as expected if *tyra-3* activity in HW is already low.

Further confirmation that the HW allele of *tyra-3* has reduced biological activity was provided by examining the one phenotype previously associated with *tyra-3*, avoidance of dilute octanol [122]. *tyra-3* null mutants avoid octanol more strongly than wild-type N2; the NIL strain with the HW *tyra-3* allele had a similar enhanced octanol response, suggesting that the HW *tyra-3* allele has reduced *tyra-3* function (**Figure 2.7** and see Appendix 2).

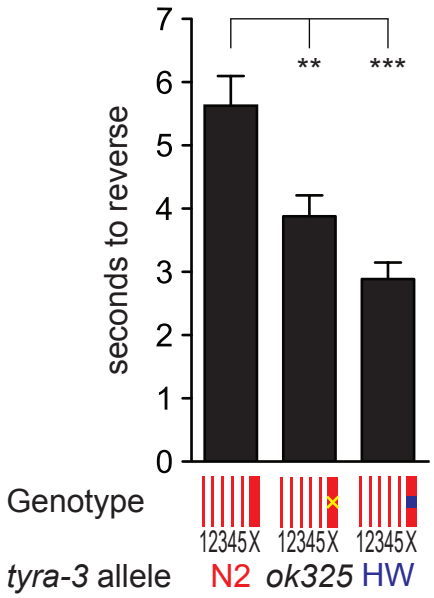

Figure 2.7

The HW allele of *tyra-3* has reduced activity in an avoidance assay. Animals were scored for avoidance of a point source of 30% octanol, off food after 40 minutes of starvation; rapid onset of reversals indicate a stronger response. Animals with a *tyra-3(ok325)* null allele or a HW *tyra-3* allele responded more rapidly than N2 animals. Error bars indicate s.e.m. ** $P < 0.01$, *** $P < 0.001$ by ANOVA with Dunnett test.

Noncoding changes affect *tyra-3* activity

The differential activity of N2 and HW genomic *tyra-3* fragments in the leaving assay suggested that N2 and HW alleles are functionally distinct (**Figure 2.4b**). To identify polymorphisms between N2 and HW alleles of *tyra-3*, I sequenced ~19 kb surrounding the *tyra-3* locus in HW. There were 34 differences between HW and the N2 consensus genomic sequence (**Figure 2.8a**): 33 noncoding changes and a single coding difference that changed a glutamate in the *tyra-3b* isoform to glycine.

Sequences that contribute to the differential activity of N2 and HW *tyra-3* alleles were localized further using transgenic assays. I fused N2 and HW *tyra-3b* cDNAs to 4.9 kb of noncoding N2 or HW sequence upstream of the *tyra-3b* start site and introduced each of the four resulting clones into the HW strain. *tyra-3* transgenes with the N2 noncoding sequence were significantly more potent than comparable transgenes with the HW sequence, regardless of whether they preceded N2 or HW *tyra-3* cDNAs (**Figure 2.8b**), excluding the coding polymorphism and localizing a functional difference between N2 and HW *tyra-3* genes to a 4.9 kb region that harbours 5 noncoding SNPs, 1 single nucleotide insertion, and a 184 bp deletion in HW. To narrow the relevant change down further, the 184 bp deletion was engineered into the N2 *tyra-3* genomic fragment; this clone was significantly less potent in the leaving assay than the full N2 genomic fragment (**Figure 2.9**). These results indicate that the 184 bp deletion

represents at least part of the functional difference between N2 and HW *tyra-3* alleles.

Sequence variation in *tyra-3* noncoding regions could affect the level or location of *tyra-3* expression. Quantitative RT-PCR of *tyra-3* mRNA levels in mixed-stage animals indicated that N2 expressed approximately twice as much *tyra-3* mRNA as HW, consistent with increased *tyra-3* activity in the N2 strain (**Figure 2.8c**). The *leav-2* NIL with N2 *tyra-3* introgressed into HW also had high *tyra-3* mRNA levels, suggesting that *cis*-acting changes affect *tyra-3* expression (**Figure 2.8c**).

Since both N2 and HW were cultivated in the laboratory for many years before permanent cultures were frozen, I wished to exclude the possibility that the *tyra-3* polymorphisms were laboratory-derived [62]. Therefore, 19 kb of the *tyra-3* locus was sequenced in all wild strains tested for leaving behavior in **Figure 2.1**, including three strains that were frozen immediately after their isolation. Each strain represents a different *C. elegans* haplotype group [43]. Both N2-like and HW-like *tyra-3* sequences were represented in the wild-caught strains, confirming the wild ancestry of both alleles (**Table 2.1** and Methods). Notably, the *tyra-3* locus of MY1 was identical to N2 and, correspondingly, the leaving rate of MY1 was similar to that of N2.

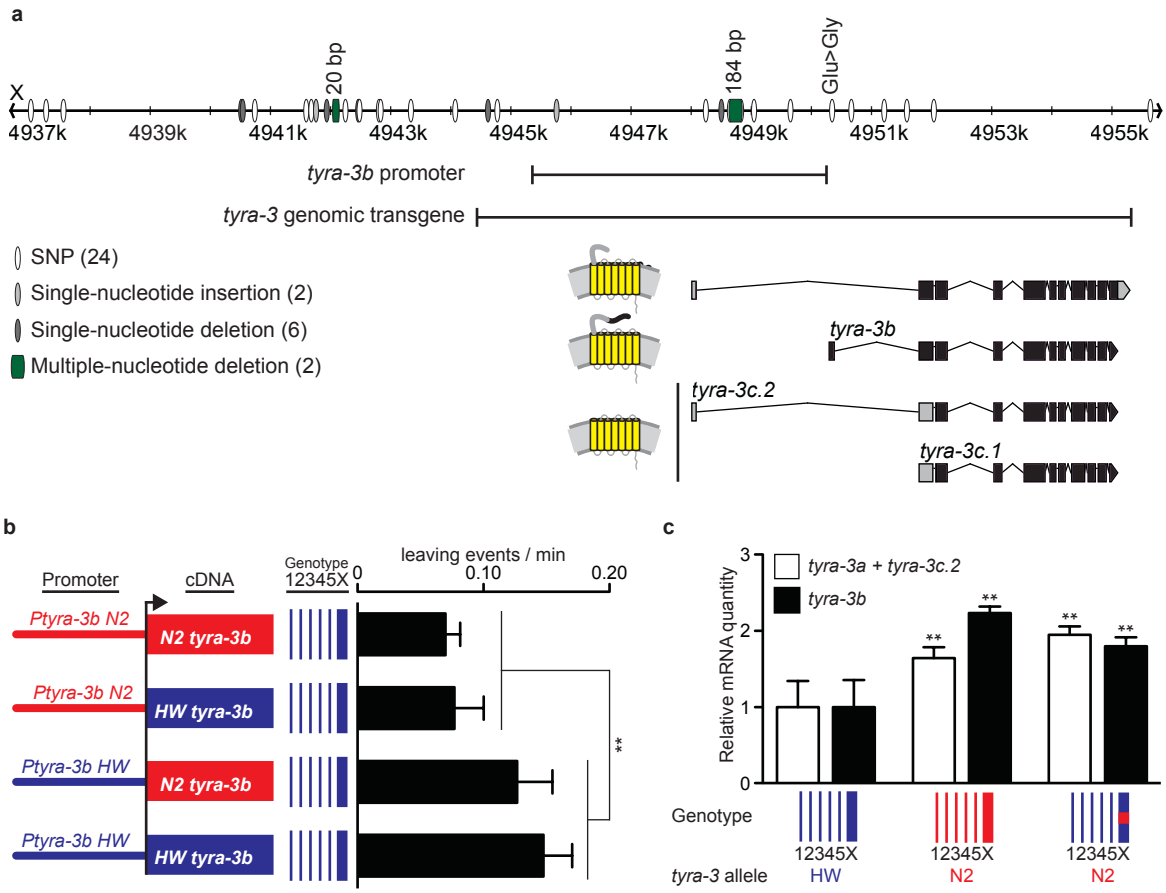

Figure 2.8

Noncoding changes in tyra-3 affect its activity and expression level. a) HW polymorphisms in the *tyra-3* locus relative to N2. *tyra-3* encodes three predicted G protein-coupled receptors. The genomic region examined in Figure 2.4b and the 4.9 kb promoter used in Figures 2.8b and 2.10a are indicated. **b)** Leaving rates of transgenic HW animals with *tyra-3b* promoters fused to *tyra-3b* cDNAs. Error bars indicate s.e.m. ** $P < 0.01$ by two-way ANOVA; no statistical interaction between the promoter and the cDNA. **c)** Relative amounts of *tyra-3* isoform mRNAs in HW, N2, and leav-2 strains (Figure 2.4a). Error bars indicate s.d. ** $P < 0.01$ compared to HW, ANOVA with Dunnett test.

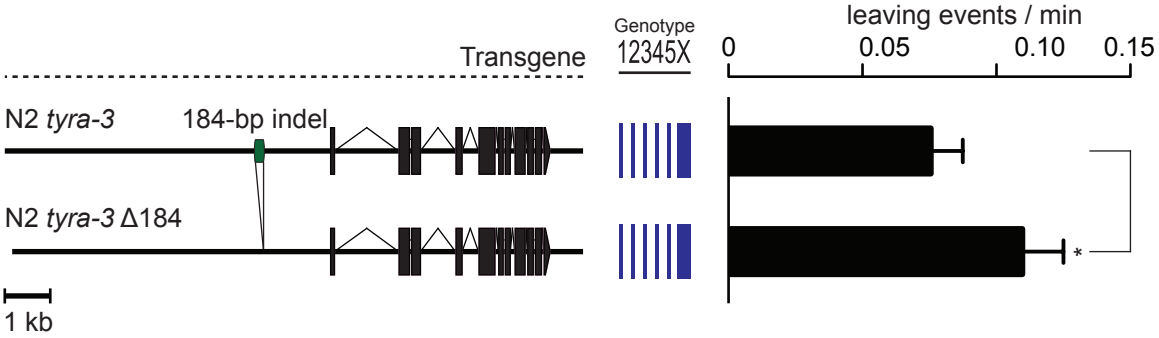

Figure 2.9

The 184 bp tyra-3 noncoding indel affects the leaving rate. Leaving rates in HW animals with an N2 *tyra-3* transgene or a similar transgene bearing an engineered 184 bp deletion. To control for variation between different transgenes, five independent transgenic lines per transgene were tested and combined. Error bars indicate s.e.m. * $P < 0.05$ by t-test.

Table 2.1. Polymorphisms in the 19 kb *tyra-3* locus

Position on X Chr. (WS210)	Wormbase reference	N2[1] (Bristol)	MY1	MY14	CB4853	JU258	CB4856 (HW)
4937008	T	T	T	C	C	C	C
4937279	G	G	G	C	C	C	C
4937525	A	A	A	C	C	C	C
4938557-4938560	4 bp[2]	Del	Del	Del	Del	Del	Del
4939032-4939033[3]	-	-	-	Ins A[4]	-	-	-
4940383	C	C	C	C	T	C	C
4940524-4940525	2 bp	2 bp	2 bp	2 bp	2 bp	Del	2 bp
4940538	A	A	A	Del	Del	A	Del
4940540	A	A	A	Del	Del	T	Del
4940740	T	T	T	C	C	C	C
4941601	A	A	A	G	G	G	G
4941668	A	A	A	A	A	C	A
4941684	A	A	A	T	T	T	T
4941752-4941753	-	-	-	Ins A	Ins A	Ins A	Ins A
4941946	A	A	A	A	A	Del	Del
4942122-4942141	20 bp	20 bp	20 bp	Del	Del	Del	Del
4942248	T	T	T	G	G	G	G
4942471	A	A	A	A	Del	A	Del
4942486	G	G	G	A	A	A	A
4942500-4942503	4 bp	4 bp	4 bp	Del	Del	4 bp	4 bp
4942565-4942573	9 bp	9 bp	9 bp	9 bp	Del	9 bp	9 bp
4942815	T	T	T	A	A	A	A
4942836	G	G	G	A	A	A	A
4943047	G	G	G	G	G	A	G
4943084	G	G	G	G	G	A	G
4943188	G	G	G	G	C	G	G
4943344	C	C	C	T	C	T	T
4944083	C	C	C	T	T	T	T
4944482	A	A	A	A	C	A	A
4944611	A	A	A	A	A	A	Del
4944629	G	G	G	A	G	G	G
4944776	A	A	A	T	T	T	T
4945282	T	T	T	G	T	T	T
4945772-4945773	-	-	-	Ins A	Ins A	Ins A	Ins A
4946063	A	A	A	A	T	A	A
4946367	T	T	T	T	T	C	T
4947025	T	T	T	T	A	T	T
4947026	G	G	G	G	A	G	G
4947027-4947028	-	-	-	-	Ins 5 bp	-	-
4947028	C	C	C	C	T	C	C

Table 2.1, *continued*

Position on X Chr. (WS210)	Wormbase reference	N2 (Bristol)	MY1	MY14	CB4853	JU258	CB4856 (HW)
4947029	G	G	G	G	C	G	G
4948269	T	T	T	A	A	A	A
4948487	A	A	A	A	A	A	Del
4948578-4948579	-	-	-	-	Ins T	-	-
4948644	A	A	A	G	G	G	G
4948657	T	T	T	C	T	C	C
4948658-4948841	184 bp	184 bp	184 bp	Del	184 bp	Del	Del
4948784	C	C	C	C	G	C	C
4948801	T	T	T	T	C	T	T
4948804-4948805	-	-	-	-	Ins T	-	-
4948807-4948808	2 bp	2 bp	2 bp	2 bp	Del	2 bp	2 bp
4949069	C	C	C	T	T	T	T
4949663	A	A	A	G	G	G	G
4950349	A	A	A	G	G	G	G
4950685	T	T	T	C	C	C	C
4951209	A	A	A	G	A	G	G
4951596	G	G	G	A	A	A	A
4952048	T	T	T	T	T	T	T
4952532	T	T	T	T	C	T	T
4952780	A	A	A	A	G	A	A
4955677	G	G	G	T	G	T	T

(1) N2 was resequenced and compared to the N2 Wormbase reference.

(2) These 4 bp are deleted in the resequenced N2 and all 'wild type' strains, indicating a discrepancy with the Wormbase reference.

(3) Insertions (Ins) lie between the two positions.

(4) The nucleotide of single-bp insertions is specified. Longer insertions are referred to by their length, indicated in parentheses.

tyra-3 acts in sensory neurons

The identification of *tyra-3* provided an opportunity to characterize the neuronal basis of the decision to leave or remain on a food patch. The biological activity of a transgene with 4.9 kb upstream of the *tyra-3b* start site fused to a *tyra-3* cDNA (**Figure 2.8b**) implied that it was expressed in cells that regulate leaving behavior. When this 4.9 kb region was fused to GFP, it drove reliable expression in ASK, ADL, AIM, AUA, BAG, CEP, OLQ, and SDQL neurons, in other unidentified neurons in the ventral ganglion and the tail, occasionally in ASH, AFD and AWC neurons, and in two non-neuronal cell types, the spermatheca and the distal tip cell (**Figure 2.10a** and data not shown). The same set of cells was observed with reporter genes bearing either N2 or HW *tyra-3* upstream regions, and in both N2 and HW genetic backgrounds. Together with the quantitative RT-PCR data (**Figure 2.8c**), these results suggest that different *tyra-3* expression levels, not different sites of expression, distinguish N2 and HW alleles.

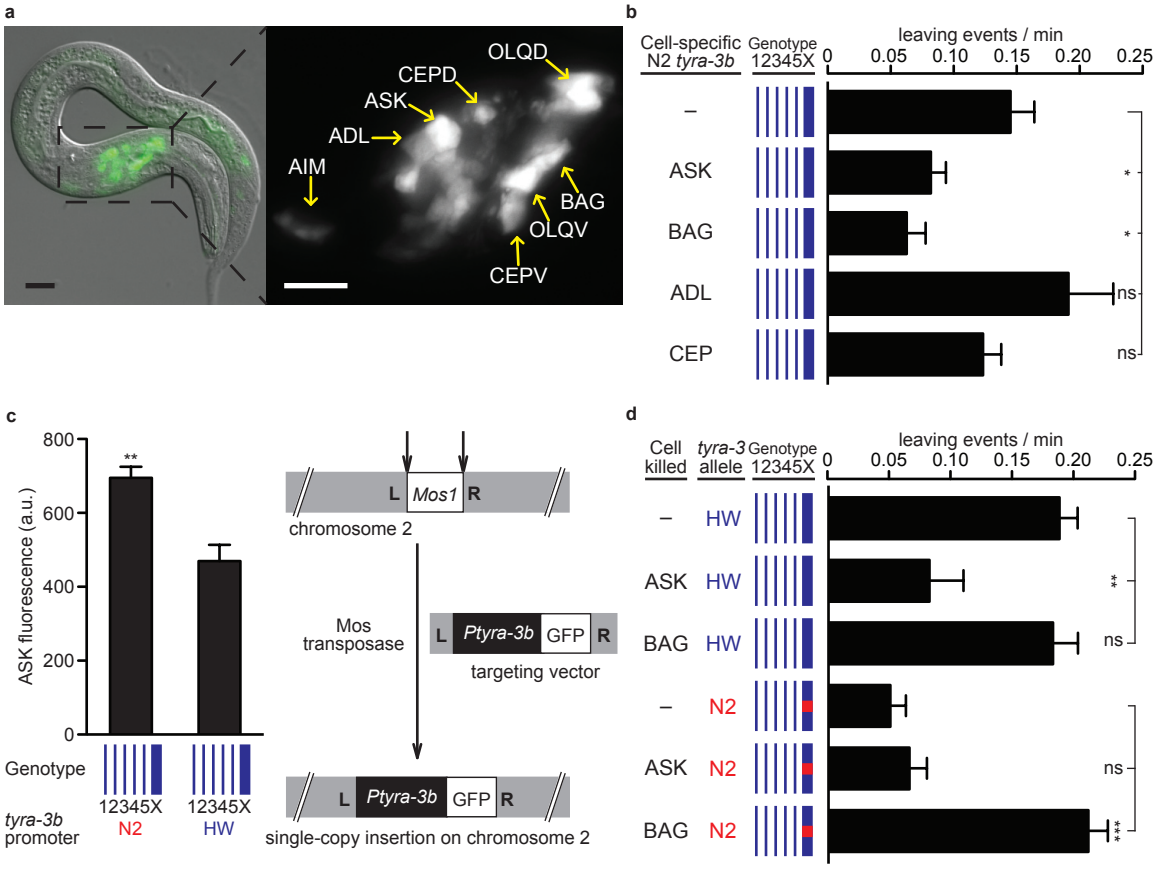

Figure 2.10

***tyra-3* acts in ASK and BAG sensory neurons. a)** Expression of 4.9 kb N2 *tyra-3b* promoter::GFP fusion (Figure 2.8a) in HW animal; HW *tyra-3b* promoter::GFP is expressed in the same cells. Posterior signal is gut autofluorescence. Scale bar = 20 µm. **b)** Leaving rates of HW strains expressing *tyra-3b* in specific cells. **c)** Left: GFP fluorescence intensity in ASK of HW animals with a MosSCI insertion of N2 or HW 4.9 kb *tyra-3b* promoter::GFP. Right: Schematic of MosSCI technique [172]. **d)** Leaving rates after killing ASK or BAG in HW and *leav-2* strains (Figure 2.4a). Error bars indicate s.e.m. * $P < 0.05$, ** $P < 0.01$, or *** $P < 0.001$ by t-test or ANOVA with Dunnett test.

The neurons whose activity is regulated by *tyra-3* were localized further by expressing *tyra-3* cDNAs from cell type-specific promoters. *tyra-3* expression in ASK or BAG sensory neurons significantly reduced leaving, but expression in the CEP or ADL sensory neurons did not (**Figure 2.10b**). The ASK neurons sense attractive food-derived amino acids [173] and regulate search behaviors after animals are removed from food [174,175]. The BAG neurons sense CO_2 and O_2, two cues associated with bacterial metabolism [176,177]. Lowering O_2 to levels that activate BAG reduced leaving rates (**Figure 2.11**).

To ask whether the *tyra-3* noncoding polymorphism affects expression in relevant neurons, single-copy N2 or HW *tyra-3b* promoters driving GFP were inserted into a single, defined chromosomal location using the MosSCI technique [172]. GFP levels in ASK neurons were significantly higher for transgenes containing the N2 promoter compared to those containing the HW promoter (**Figure 2.10c**). These results suggest that the N2 *tyra-3* locus is associated with higher *tyra-3* expression in ASK, as well as higher *tyra*-3 mRNA expression at a whole-animal level; expression in BAG was not examined.

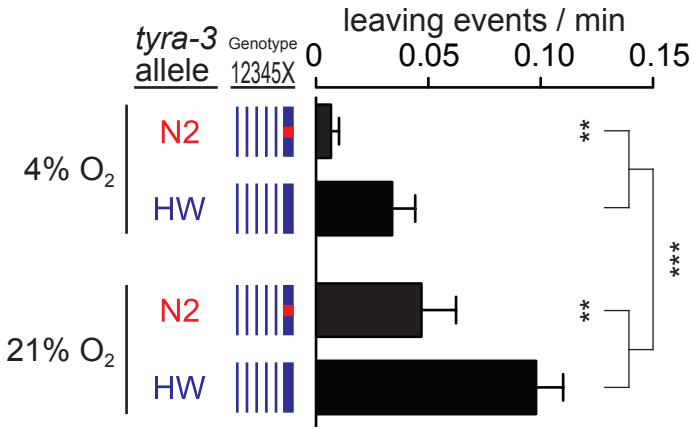

Figure 2.11.

Lowering O_2 levels reduces leaving rates. HW animals with either N2 or HW alleles of *tyra-3* have lower leaving rates at 4% O_2 than at 21% O_2. Assays were conducted in a flow chamber with oxygen concentrations controlled by external tanks of mixed gases. Two-way ANOVA showed significant effects of both the *tyra-3* genotype and the O_2 concentration, with no significant interaction between them. Error bars indicate s.e.m. ** $P < 0.01$, *** $P < 0.001$ by two-way ANOVA.

The behavioral functions of ASK and BAG, and *tyra-3*'s effect on those functions, were assessed by killing the neurons in different genetic backgrounds. Killing the ASK neurons reduced the leaving rate of HW animals, indicating that ASK can promote leaving (**Figure 2.10d**). The ablation resembled the effect of the ASK::*tyra-3* transgene, suggesting that *tyra-3* reduces ASK activity. In agreement with this idea, killing the ASK neurons in a strain with the N2 high-activity *tyra-3* allele did not reduce their leaving rates further. The effect of *tyra-3* on ASK was selective for this assay; *tyra-3* did not reduce lysine chemotaxis, a second ASK-dependent behavior (**Figure 2.12**).

Killing the BAG neurons increased leaving rates in the strain with the N2 *tyra-3* allele, demonstrating that BAG neurons prevent leaving (**Figure 2.10d**). However, killing BAG had no effect in the strain with the HW *tyra-3* allele, suggesting that BAG activity is already low in this strain under the assay conditions. The ablation and genetic results suggest that the N2 *tyra-3* allele decreases ASK activity and increases BAG activity, two changes that act together to prevent leaving (**Figure 2.13**).

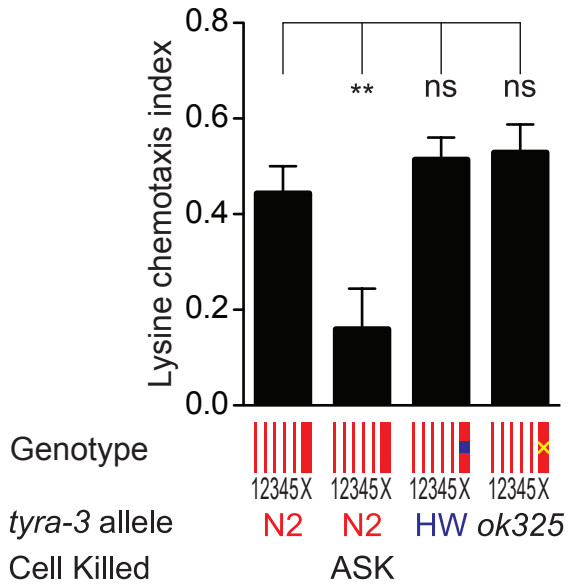

Figure 2.12

***tyra-3* does not affect lysine chemotaxis.** Killing ASK reduces chemotaxis to 5 mM L-lysine, but a *tyra-3(ok325)* null allele or a HW *tyra-3* allele did not affect the behavior. Chemotaxis was scored by adding lysine to two quadrants of agar on a small plate, placing washed animals in the center of the plate, and examining their distribution after 5 minutes; chemotaxis index = [(animals on lysine)-(animals not on lysine)]/(total number of animals). Error bars indicate s.e.m. ** $P < 0.01$ by ANOVA with Dunnett test.

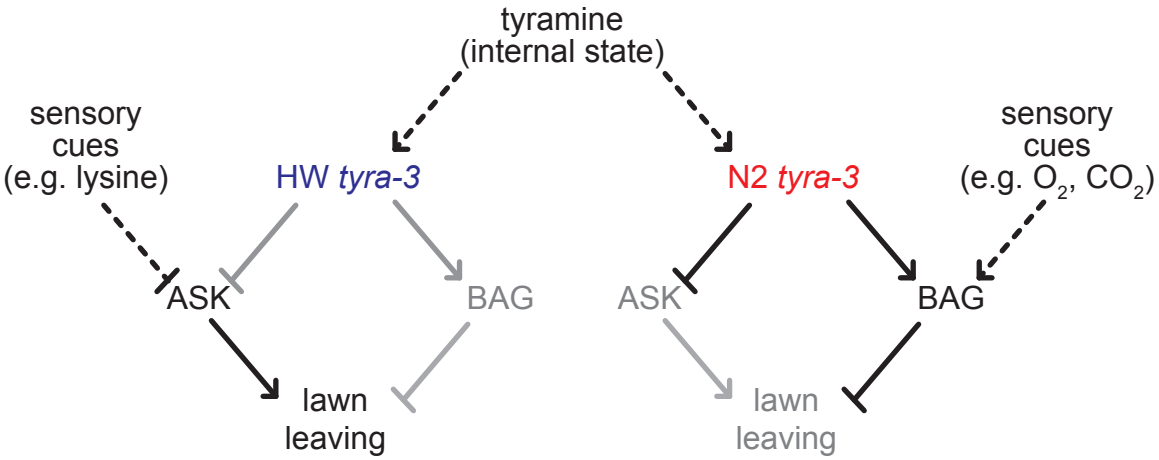

Figure 2.13

***tyra-3* polymorphism affects an exploration-exploitation decision.** The *tyra-3* gene senses tyramine and modifies the function of ASK and BAG chemosensory neurons, which promote or inhibit lawn-leaving, respectively. The N2 high-activity allele of *tyra-3* suppresses the function of ASK neurons and enhances the function of BAG neurons compared to the HW low-activity allele of *tyra-3*. Both functions of the N2 *tyra-3* allele suppress lawn-leaving. Solid lines indicate activities identified in this study; dashed lines indicate results from prior studies.

tyra-3 is a tyramine receptor

Biochemical evidence shows that *tyra-3* binds tyramine with nanomolar affinity and this binding is competed 10 times more strongly by tyramine than by octopamine [122]. Moreover, *tyra-3* is required for some behavioral responses to exogenous tyramine, but not required for responses to exogenous octopamine. To confirm that tyramine is the endogenous ligand for *tyra-3*, I tested whether behavioral changes induced by *tyra-3* overexpression are suppressed by mutants that are unable to synthesize tyramine. Transgenic *tyra-3* overexpression reduces the leaving rate of HW animals (**Figure 2.4b**) and also suppresses their social behavior (**Figure 2.14**). The reduced social behavior of HW worms overexpressing *tyra-3* is completely suppressed by a loss of function mutation in *tyrosine decarboxylase 1 (tdc-1)*, which is required for tyramine and octopamine biosynthesis (**Figure 2.14** and **Figure A2.3a**). In contrast, a loss of function mutation in *tyramine β-hydroxylase 1 (tbh-1)*, which is required for the conversion of tyramine into octopamine (**Figure A2.3a**), does not impact the reduced social behavior induced by *tyra-3* overexpression (**Figure 2.14**). In concert with the biochemical results and pharmacological manipulations, these genetic experiments point to tyramine as an important ligand for *tyra-3*. Future experiments will determine whether tyramine acts as *tyra-3*'s ligand to modify the patch leaving rate; preliminary results are shown in Appendix 2.

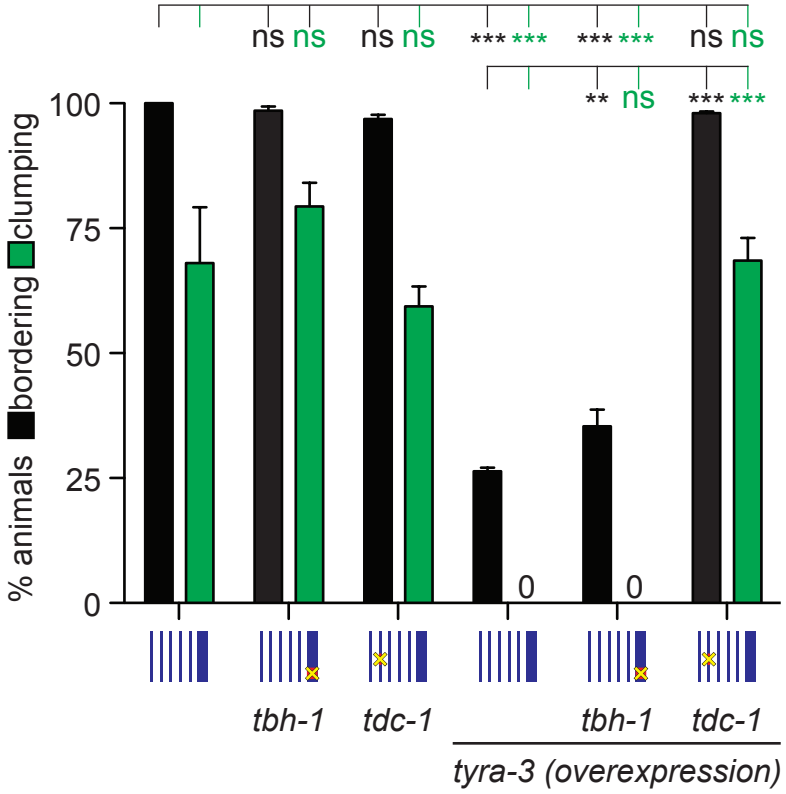

Figure 2.14

Tyramine is an endogenous ligand for *tyra-3***.** Bordering and clumping behaviors of HW animals and HW animals with a *tbh-1(n3247)* and *tdc-1(n3420)* in the absence or presence of transgenic overexpression of a genomic *tyra-3* fragment (Figure 2.8a). *tbh-1(n3247)* and *tdc-1(n3420)* were introgressed into HW from an N2 background. Error bars indicate s.e.m. ** $P < 0.01$, *** $P < 0.001$ by ANOVA with Bonferroni test.

Gene-gene-environment interactions

Like most natural behaviors, the decision to leave a food patch is regulated by multiple genes and the environment; it responds to genetic variation in *tyra-3, npr-1,* and additional genes on the autosomes (**Figure 2.1**) as well as food quality and quantity [121,162]. Our results suggested that the N2 *npr-1* allele was epistatic to *tyra-3*; animals with the N2 *npr-1* allele had low leaving rates regardless of the *tyra-3* genotype (**Figure 2.4a**). However, N2 *npr-1* reduced the leaving rate to almost zero, making it difficult to detect any further reduction. To make the assay more powerful, leaving was assayed on bacterial lawns of different densities. Leaving rates of all genotypes increased on thinner lawns and decreased on thicker lawns (**Figure 2.15**), but the thickness of the lawn changed the genetic interaction between *tyra-3* and *npr-1*. In the standard leaving assay, *tyra-3* polymorphisms had different effects only in the presence of the HW *npr-1* allele; on a thinner lawn, only in the presence of the N2 *npr-1* allele (**Figure 2.15**). Thus the epistatic relationship between *npr-1* and *tyra-3* is defined by the specific environment, not by an intrinsic regulatory relationship between the genes.

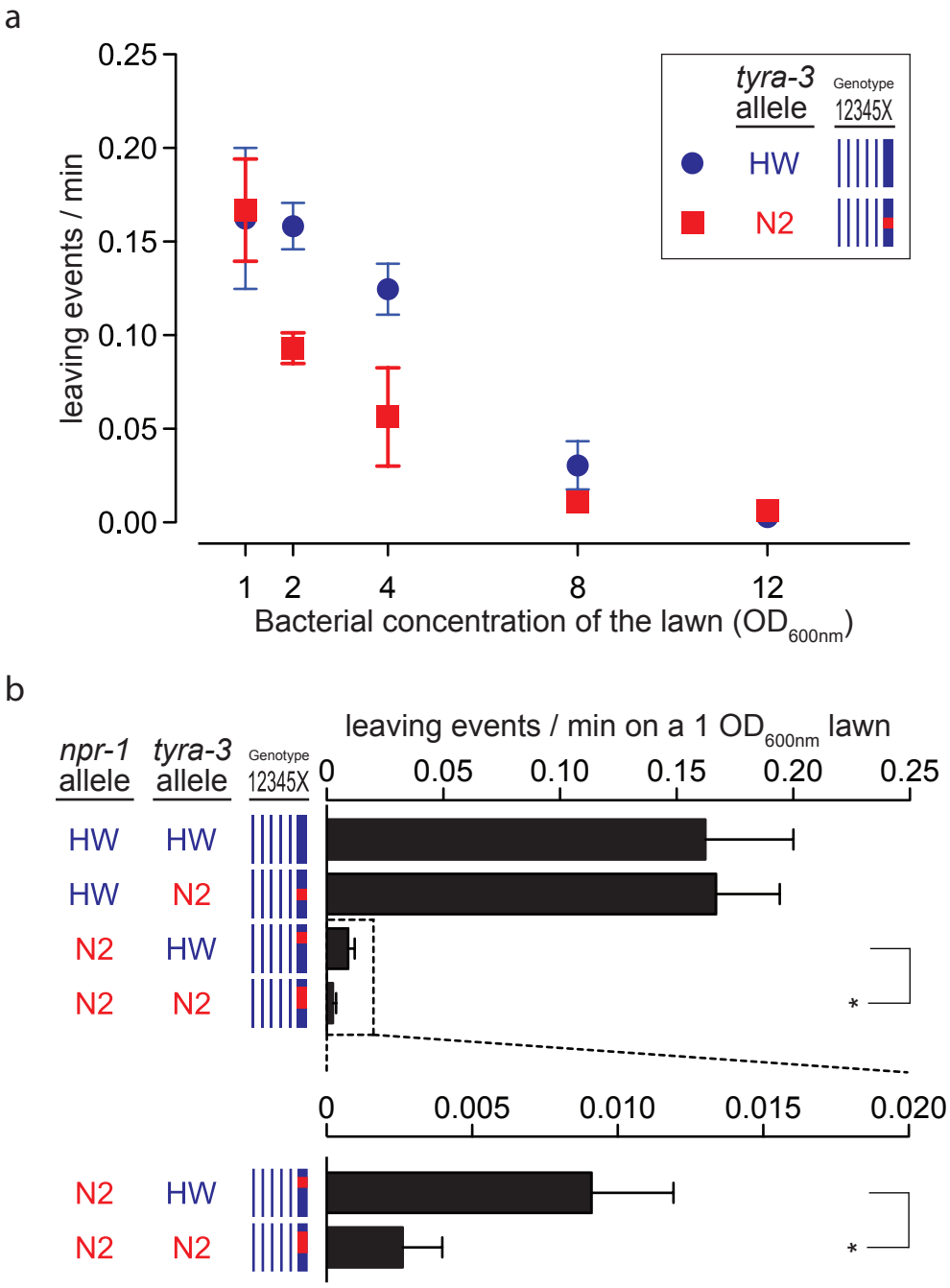

Figure 2.15

Density of the bacterial lawn affects leaving rates and genetic interactions between *npr-1* and *tyra-3*. **a)** Leaving rates of both N2 *tyra-3* and HW *tyra-3* strains (HW *npr-1* background) are higher on thinner lawns. **b)** N2 *tyra-3* further decreases the leaving rate of N2 *npr-1* on OD_{600nm}=1 lawns, but not on OD_{600nm}=2 lawns (Figure 2.4a). Error bars indicate s.e.m. * $P < 0.05$ by t-test.

Discussion

Our results show that natural variation in *tyra-3* affects patch leaving, a behavior representative of the exploration-exploitation decision. *tyra-3* encodes a G protein-coupled receptor activated by the invertebrate transmitter tyramine and less potently by octopamine [122], which is structurally related to vertebrate epinephrine and norepinephrine. Monoamines are known to regulate arousal systems that affect many behaviors and behavioral decisions. In *C. elegans,* octopamine drives sensory, molecular, and behavioral responses to starvation, and tyramine affects specific aspects of locomotion [178-181]. In insects, octopamine acts as a reward-related signal during learning, and also affects locomotory activity, arousal, and aggression [124,182-184]. Mammalian norepinephrine is generally implicated in arousal behaviors, and norepinephrine release from the locus coeruleus is associated with switching between different tasks, a cognitive function with analogies to the exploration-exploitation decision [125]. The central nervous system effects of norepinephrine are mediated mainly by adrenergic receptors related to *tyra-3* [185]

Relatively few natural behavioral variations have been mapped to the single-gene level in any animal, and it is interesting that several of these variations affect G protein-coupled receptor signaling systems. In addition to *tyra-3*, a mouse behavioural QTL for increased anxiety corresponds to *Rgs2*, a negative regulator of G protein signalling [79], and in voles, interspecies variation in social behaviour is associated with differential expression of the G protein-

coupled receptor for the neuropeptide arginine vasopressin [136]. In humans, DRD4 dopamine receptor variation is associated with novelty-seeking behavior [146]. I speculate that these receptor pathways may serve as common substrates of behavioral variation. All animal genomes encode many G protein-coupled receptors with different expression patterns. For example, the *C. elegans* genome encodes at least six G protein-coupled receptors for tyramine or octopamine and one ligand-gated tyramine channel. These receptors may provide a reservoir for genetic changes, as alteration in an individual receptor could cause relatively discrete effects without disrupting the entire system.

QTL mapping in rodents and in *Drosophila* indicates that most behavioral traits are polygenic, with widespread epistatic effects [157,186]. In agreement with this conclusion, our analysis suggests the existence of epistatic interactions between *tyra-3, npr-1,* and at least one additional locus. Importantly, the non-additive interactions between *tyra-3* and *npr-1* are not stable, but vary based on the genetic background and the environment. A similar conclusion has emerged from a comprehensive study of yeast sporulation, where epistatic interactions between four genetic variants are highly sensitive to environmental conditions and genetic background [187].

By integrating genetic studies of *C. elegans* foraging with neuronal analysis, a first-level description of underlying mechanisms emerges. The sensory neurons that express *tyra-3* detect food-related cues; I suggest that they integrate these external cues with internal arousal states detected by *tyra-3*, and

that different *tyra-3* alleles confer differential sensitivity to these arousal states (**Figure 2.13**). *C. elegans* patch-leaving is strongly affected by aversive (arousing) cues [163,164], so variation in arousal systems is a plausible basis for the variable patch-leaving behaviour described here. Thus variation in *tyra-3* lies at the intersection of many forms of behavioral flexibility: rapid responses to environmental cues, short-term modulation by internal state fluctuations, and long-term genetic changes that lead to adaptive changes in innate behaviors.

"Animals are very literal; they live in a world of truisms."

–G.K. Chesterton

CHAPTER 3: GABA receptor polymorphisms modify social behavior in *C. elegans*

Introduction

Social interactions are pervasive in the animal kingdom: communication, reproductive behavior, agonistic actions, and affiliative behavior play crucial roles in the lives of most animals [188]. While social interactions are prevalent in most animal species, these interactions vary greatly in form and magnitude both within species and between closely related species. For example, closely related species of voles differ drastically in pair-bonding and parental behaviors [189-191]. In voles, interspecific differences in the neuronal expression pattern of the V1a vasopressin receptor contribute to variation in pair-bonding behavior [139]. The genetic changes that contribute to variation in V1a expression pattern or to social behavior differences in other species are largely unknown.

Aggregation between members of a species is a simple form of social interaction. Wild strains of *Caenorhabditis elegans* are social feeders and aggregate in the presence of food [61,62]. This social behavior of *C. elegans* is partly a defensive strategy: animals effectively lower high surrounding oxygen

levels – which they dislike – by aggregating with other animals [168]. Pheromones released by other animals also play a role in *C. elegans* social behaviors [192,193,133].

During the laboratory domestication of *C. elegans*, the common laboratory strain N2 evolved to be a solitary feeder. Two mutations are responsible for the development of solitary behavior in laboratory *C. elegans*. A loss-of-function mutation in the sensory globin gene *glb-5* affects the ability of animals to detect small oxygen changes, while a gain-of-function mutation in the neuropeptide Y receptor homologue *npr-1* modifies a circuit that integrates attractive and repulsive cues, including pheromones [61,133,62,106]. It is not known, however, whether these genes are the only modifiers of social behavior that differ between wild type *C. elegans* strains.

To further understand the genetics of social behavior, I used quantitative genetic techniques to identify additional social genes and found that noncoding polymorphisms in the GABA receptor *exp-1* contribute to variation in social behavior.

Multiple loci modulate social behavior

Social *C. elegans* strains display a constellation of distinctive behaviors: they prefer the border of a bacterial lawn on which they feed over the center, they aggregate with other animals at the border of the bacterial lawn, and they move fast when traversing the lawn [61]. In contrast, animals from solitary strains

distribute themselves randomly on a bacterial lawn, do not aggregate with other animals, and move slowly on food [61].

Variation in a single amino acid of the neuropeptide Y receptor homologue *npr-1* mediates most of the difference in social behavior between solitary and social strains of *C. elegans.* Social strains like CB4856 from Hawaii (HW) have a phenylalanine at position 215 of *npr-1* while solitary strains have acquired a mutation at 215 that encodes valine [61]. Replacing the derived *npr-1* allele of the solitary N2 strain with the ancestral HW allele of *npr-1* from a social strain in a near-isogenic line (NIL) significantly transforms the solitary behavior of N2 toward HW-like social behavior [61,62] (Bordering and aggregation quantification in **Figure 3.1a**). While a HW *npr-1* allele in an N2 background promotes substantial levels of social behavior, the necessity for a HW allele of *npr-1* in the HW background has not been explored.

To determine whether a HW allele of *npr-1* is required for social behavior, I substituted the N2 allele of *npr-1* for the HW allele in a HW strain through introgression. Although HW animals with N2 *npr-1* showed significantly less bordering and aggregation (clumping) behaviors than HW animals, their social behavior was intermediate between HW and N2, suggesting that variation in genes other than *npr-1* promote social behavior in the HW strain (**Figures 3.1a** and **3.1b**).

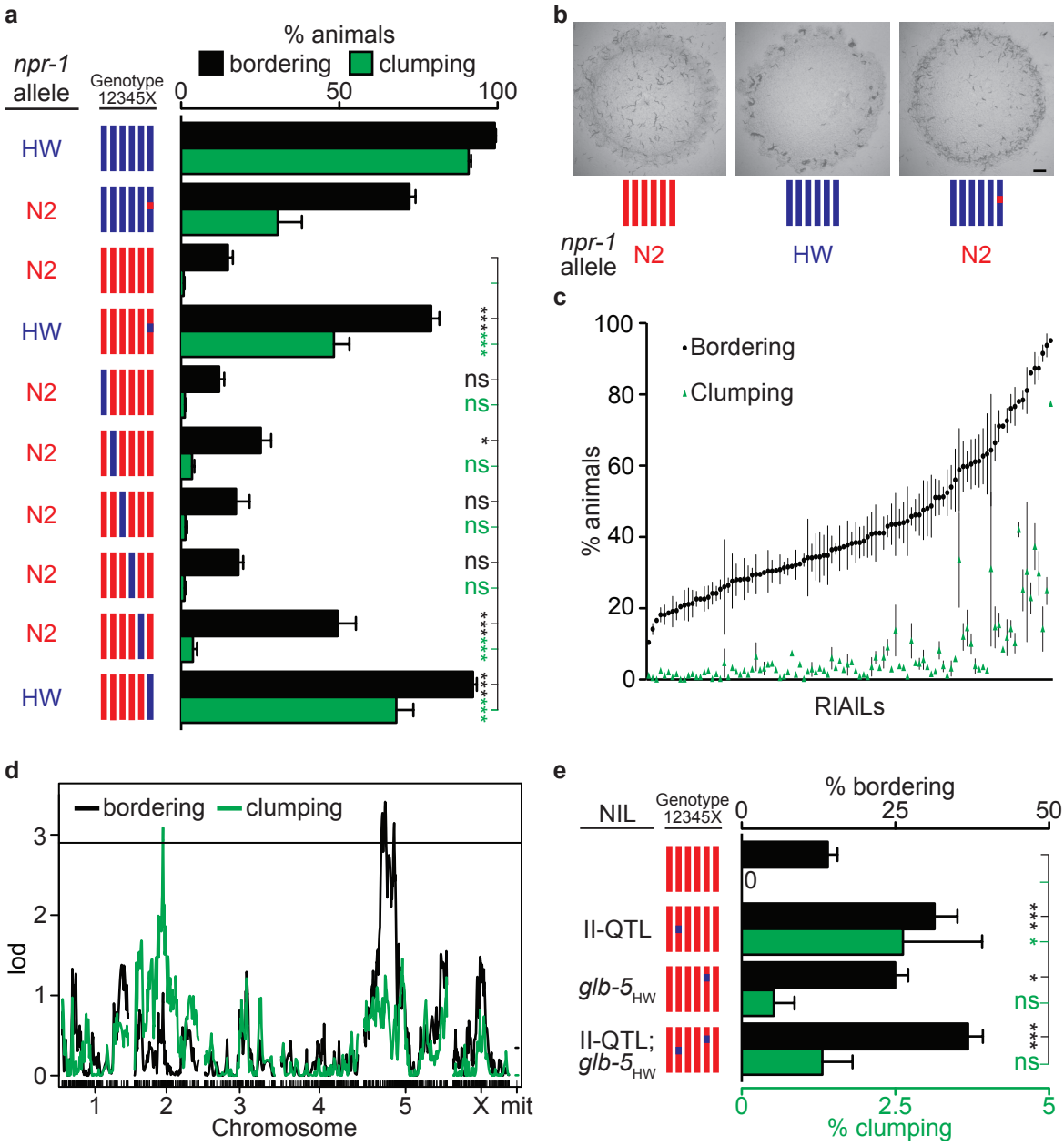

Figure 3.1

Two autosomal QTL for social behavior in *C. elegans*. **a)** Social behaviors of *npr-1* near-isogenic lines (NILs) and of chromosome-substitution strains. **b)** Solitary behavior of N2 animals (left), and social behavior of HW animals (middle) and HW animals with N2 *npr-1* (right). Scale bar, 2 mm. **c)** Social behaviors of 102 N2-HW recombinant inbred advanced intercross lines (RIAILs) with N2 *npr-1*. **d)** QTL analysis of RIAILs shown in **c.** The horizontal line denotes the $P < 0.05$ genome-wide significance threshold. lod, log likelihood ratio. **e)** Social behaviors of NILs with the QTL identified on **d.** Error bars in **a**, **c**, and **e**, s.e.m. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, by ANOVA with Bonferroni test.

I performed quantitative genetic analysis to identify the loci that mediate differences in social behavior between the N2 and HW *C. elegans* strains. Two independent approaches identified social behavior loci on chromosomes II and V. Chromosome substitution strains in which each of the six N2 chromosomes were individually replaced by a HW chromosome showed that HW chromosomes II, V and X have social behavior loci (**Figure 3.1a**). The social behavior of the chromosome X substitution strain is higher than the NIL containing HW *npr-1* in an N2 background, suggesting that additional loci on chromosome X modulate social behavior (**Figures 3.1a**).

In an orthogonal approach, quantitative trait locus (QTL) analysis was performed on 102 recombinant inbred advanced intercross lines (RIAILs) derived from crosses between N2 and HW [43]. To facilitate the identification of loci other than *npr-1*, only RIAILs with the N2 allele of *npr-1* were tested for social behavior. When a single QTL affects a trait in an inbred mapping population, the distribution of trait values should be bimodal in that population. By contrast, the RIAILs showed a unimodal distribution for both bordering and aggregation behaviors, suggesting that multiple QTLs affect social behavior (**Figure 3.1c**). Moreover, while bordering and aggregation behaviors were correlated (R^2=0.53, P<0.0001) in the RIAILs, this correlation was far from perfect, suggesting that the genetics of bordering and clumping are similar, but partially non-overlapping.

QTL analysis identified a significant QTL on chromosome V (V-QTL) for bordering behavior and a significant QTL on chromosome II (II-QTL) for clumping

behavior (**Figure 3.1d**). The location of these QTLs agrees with the findings of the chromosome substitution strains (**Figure 3.1a**). The V-QTL overlaps *glb-5*, confirming *glb-5*'s involvement in social behavior variation between N2 and HW. The chromosome V substitution strain, however, has a stronger social phenotype than a NIL containing HW *glb-5* in an N2 background, suggesting that other loci on chromosome V in addition to *glb-5* modify social behavior (**Figures 3.1a** and **3.1d**).

Consistent with the inference of QTL additional to the II-QTL and *glb-5*, a NIL containing HW II-QTL and HW *glb-5* in an N2 background was not as social as a HW strain with an N2 allele of *npr-1* (**Figure 3.1e**, note difference in x-axis scale from **Figure 3.1a**). The aggregation behavior of the HW II-QTL; HW *glb-5* double strain was particularly mild compared to the HW strain with the N2 *npr-1* allele and was even less pronounced than that of the II-QTL strain. A reduction of clumping behavior induced by a HW allele of *glb-5* has previously been observed in N2 animals with HW *npr-1* [62]. Thus the HW allele of *glb-5* may either promote or reduce aggregation depending on the genetic background.

Fine-mapping and origin of the social behavior II-QTL

To fine-map the chromosome II social behavior QTL, the HW II-QTL in an N2 background was dissected through recombination with N2 (these experiments were done together with a rotation student, Jason Pitts). An analysis of 5,000 recombinants identified a 6.2 kb interval as the minimal region containing the II-

QTL (**Figure 3.2a**). This 6.2 kb interval was fully contained within a single gene, *abts-3*, an anion transporter (**Figure 3.2b**).

Sequencing the region containing the QTL in N2 and HW uncovered 11 polymorphisms (**Figure 3.2** and **Table 3.1**). Compared to N2 there were six noncoding single nucleotide polymorphisms (SNPs), one coding SNP (*abts-3a* G615D), one single nucleotide deletion, one single nucleotide insertion, a three-nucleotide insertion and a 23-nucleotide deletion in HW. Since mutations in the *npr-1* and *glb-5* genes that affect social behavior arose during the laboratory domestication of the N2 strain [62], I wished to determine whether the II-QTL had also evolved as a laboratory adaptation of the N2 strain. LSJ2, a sister strain of N2, was separated early in the initial laboratory cultivation of N2, 50 years ago, and maintains the ancestral alleles of *npr-1* and *glb-5* [62]. LSJ2 did not vary from N2 in the sequence of the II-QTL, indicating that N2 has not acquired mutations in the II-QTL since its separation from LSJ2. In fact, sequencing the II-QTL in four additional wild *C. elegans* strains that represent different haplotypes [43] revealed that N2 has only two SNPs that are not present in other strains (**Figures 3.2b** and **3.2c** and **Table 3.1**). These two SNPs could have occurred before N2 was brought to the laboratory or in captivity before its divergence with LSJ2.

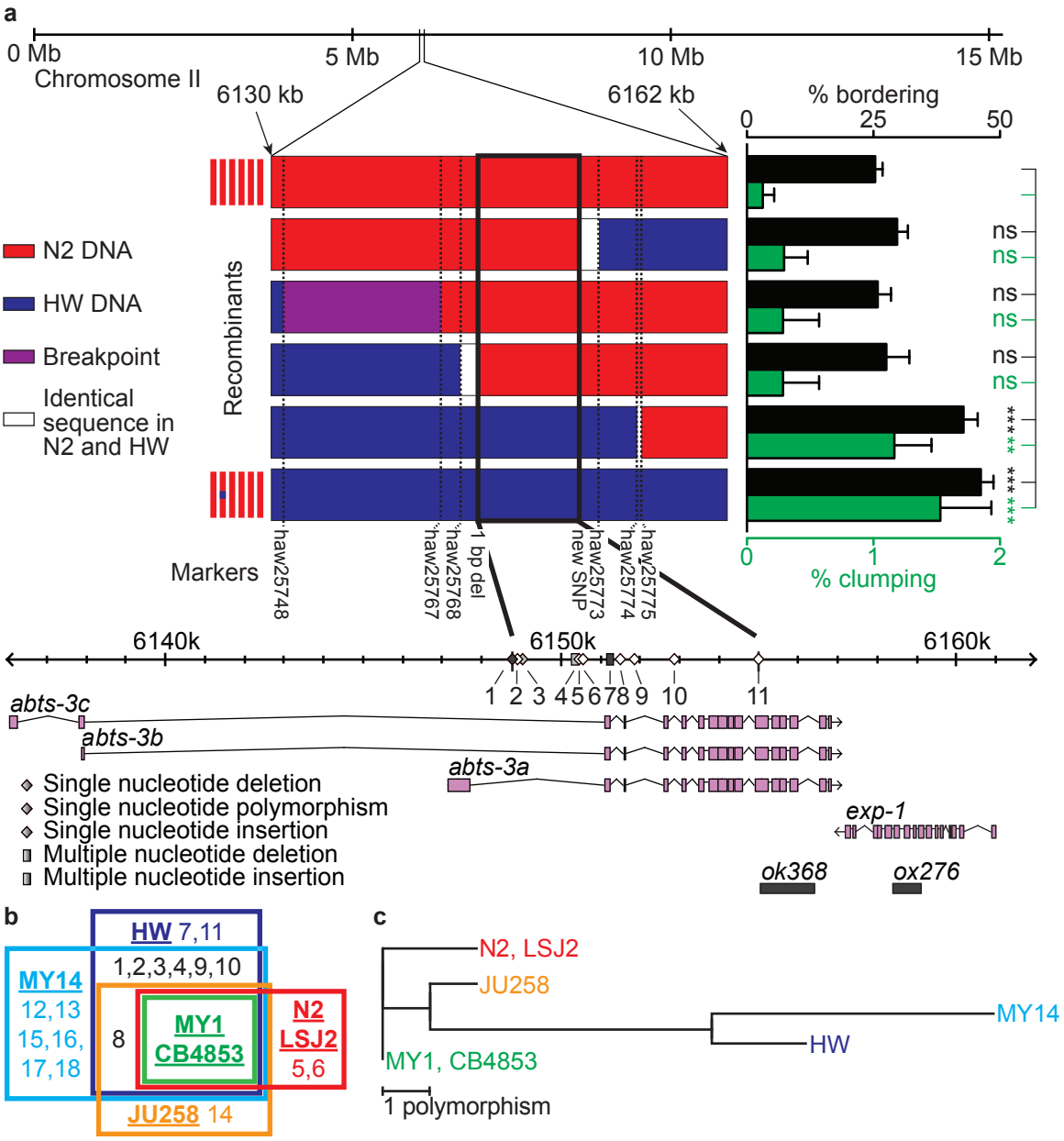

Figure 3.2

Fine-mapping a social behavior QTL to a 6.2 kb region. **a)** Top: bordering and clumping behaviors of recombinants in the II-QTL region in an N2 background. Polymophisms used for genotyping are shown under recombinants. Bottom: expansion of the 6.2 kb QTL, showing polymorphisms between N2 and HW, protein coding genes, and deletion alleles used in Figs. 3.3-3.6. **b)** Polymorphisms in the QTL region of wild-type strains relative to the consensus sequences of MY1 and CB4853. Shared polymorphisms are in black and in regions of overlap between boxes. **c)** Phylogenetic three of the II-QTL with data from **b**. Error bars, s.e.m. ** $P < 0.01$ by ANOVA with Dunnett test.

Table 3.1 Polymorphisms in the 6.2 kb chromosome II QTL

Position on Chr. II (WS226)	Polymor-phism #	MY1 & CB4853	N2[(1)] & LSJ2	MY14	JU258	CB4856 (HW)
6148781[(2)]	1	A	A	Del	A	Del
6148900	2	T	T	G	T	G
6149014-6149015[(2)]	3	-	-	Ins T	-	Ins T
6150458-6150459	4	-	-	Ins TCA	-	Ins TCA
6150465	5	C	A	C	C	C
6150559	6	C	A	C	C	C
6150634	12	C	C	T	C	C
6151354	13	G	G	C	G	G
6151494	7	C	C	C	C	A
6151791-6151792	14	-	-	-	Ins T	-
6151873	8	C	C	T	T	T
6152099-6152121	9	23 bp	23 bp	Del	23 bp	Del
6152280	15	C	C	A	C	C
6152281	16	A	A	C	A	A
6152858	10	T	T	G	T	G
6153301	17	C	C	G	C	C
6154296	18	A	A	G	A	A
61549909[(3)]	11	A	A	A	A	G

(1) N2 was resequenced and no differences were found with the N2 Wormbase reference.

(2) Insertions (Ins) lie between the two positions.

(3) This is the only nonsynonymous substitution in the QTL (*abts-3a* G615D)

The GABA receptor *exp-1* affects social behavior

Since the II-QTL was fully contained within the *abts-3* gene, a likely hypothesis was that different *abts-3* activity between N2 and HW affects social behavior. To test this idea I determined the activity of a loss of function mutation in *abts-3* in a quantitative complementation test with the II-QTL [71]. I first determined that an N2 II-QTL / HW II-QTL heterozygote has an intermediate phenotype, but more closely resembles the N2 II-QTL homozygote (**Figure 3.3a**). This dominance relationship suggested that the N2 II-QTL has higher activity than the HW II-QTL and that a mutant *abts-3* gene should fail to complement the HW II-QTL or increase the HW II-QTL social phenotype. A deletion allele of *abts-3(ok368)* in an N2 background, however, complemented the HW II-QTL: the social behavior of a HW II-QTL / *abts-3(ok368)* heterozygote was significantly different from a HW II-QTL homozygote, but not significantly different from an N2 II-QTL homozygote, inconsistent with the notion that variation in *abts-3* activity affects social behavior (**Figure 3.3a**).

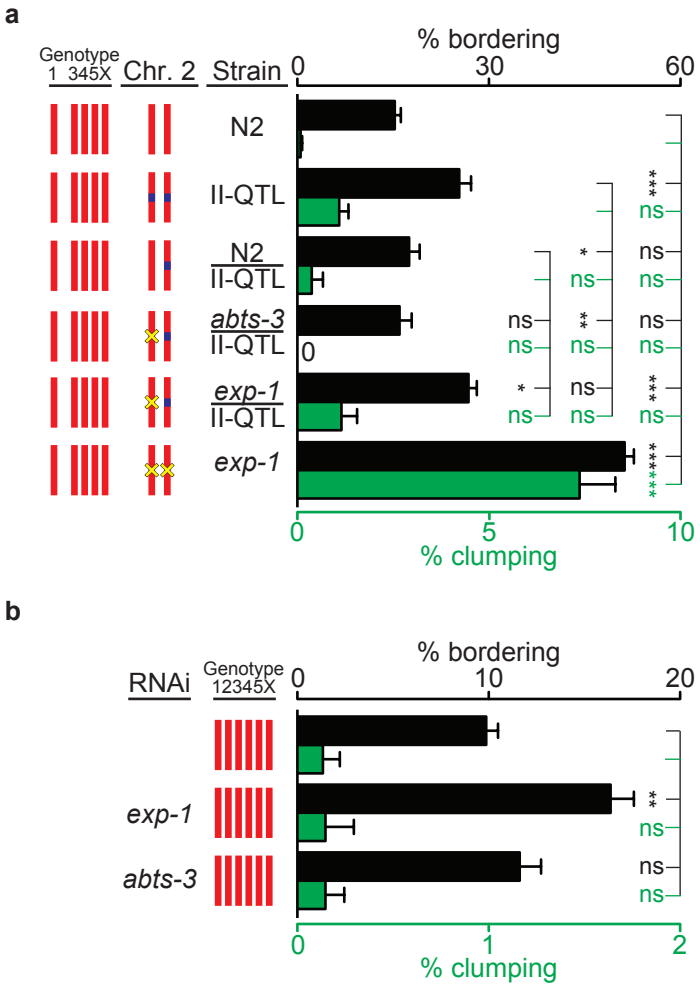

Figure 3.3

***exp-1* is a social quantitative trait gene.** **a)** Complementation tests between the HW II-QTL, N2, and deletion mutants of *abts-3(ok368)* and *exp-1(ox276)*. **b)** Social behavior after RNAi knock-down of *exp-1* and *abts-3*. Error bars, s.e.m. ** $P < 0.01$, *** $P < 0.001$ by ANOVA with Bonferroni or Dunnett tests.

The ability of a loss of function allele of *abts-3* to complement the low-activity HW II-QTL suggests that variation in the II-QTL affects a different gene. After *abts-3,* the nearest gene to the II-QTL is *exp-1*, which codes for a γ-amino butyric acid (GABA)-gated cation channel [194]. The stop codon of *exp-1* is 2.2 kb distal from the 6.2 kb QTL (**Figure 3.2**); the next gene in the region is *hst-3.1*, a heparan sulfotransferase, located 13 kb away. There is precedent in *C. elegans* for transcriptional regulatory regions located as far as 5.6 kb 3' to the stop codon of a gene [195], so it is conceivable that the II-QTL affects the expression of *exp-1*.

In contrast with the ability of an *abts-3* mutation to complement the HW II-QTL, a loss of function mutation in *exp-1(ox276)* failed to complement the reduced activity HW II-QTL (**Figure 3.3a**). In addition, the homozygous null *exp-1(ox276)* mutant exhibited a substantial degree of aggregation and bordering. These results suggest that HW II-QTL harbors a reduced activity allele of *exp-1* compared to N2. If this is true, reducing *exp-1* activity in the N2 strain that has higher-activity of *exp-1* should mimic the HW II-QTL and lead to increased social behavior. Indeed, RNAi against *exp-1* in the N2 strain increased bordering behavior, whereas RNAi against *abts-3* did not affect the social behavior of N2 (**Figure 3.3b**). These results are consistent with *exp-1*, and not *abts-3*, being the social behavior gene affected by the II-QTL.

The social behavior of HW II-QTL animals is intermediate between N2 and the homozygous null allele *exp-1(ox276)* (**Figure 3.3a**). Moreover, the HW II-QTL complements the defecation defects of *exp-1(ox276)*, suggesting that the

HW II-QTL provides full *exp-1* function in the defecation cycle (**Figures 3.3a** and **3.4**). Therefore, the HW II-QTL corresponds to a reduced or partial activity allele of *exp-1*, not to a complete loss of function allele.

Interactions between *exp-1* and other GABA pathway mutants suggest that GABA both promotes and suppresses social behavior

Since EXP-1 has been shown to be one of multiple GABA receptors in *C. elegans*, I next examined other mutations in the GABA pathway for their effects on social behavior. The increased social behavior of the *exp-1(ox276)* null allele indicates that this GABA receptor normally antagonizes social behavior. If GABA acts solely through *exp-1* to decrease social behavior, animals with mutations that eliminate GABA transmission should have a similar increase in social behavior. I tested this hypothesis by measuring the social behavior of animals with a loss of function mutation in the vesicular GABA transporter *unc-47*. Animals with an *unc-47(e307)* mutation are defective in all *C. elegans* behaviors that require GABA: backward motion, foraging behavior, and defecation [196,197]. By contrast, social behavior in *unc-47(e307)* animals was identical to that of wild-type N2 animals. There are two possible explanations for these results: *exp-1* could have a GABA-independent function, or the *unc-47* mutation could eliminate GABA effects mediated through *exp-1* together with antagonistic effects mediated through another GABA receptor. In agreement with the latter possibility, an *exp-1(ox276); unc-47(e307)* double mutation resembled *unc-47*

rather than *exp-1* in its social behavior (**Figure 3.5a**). These results suggest that GABA, in addition to inhibiting social behavior through *exp-1*, also promotes social behavior through additional receptors (**Figure 3.5b**).

Bordering and clumping of *exp-1* mutants require pheromones

Aggregation is associated with alterations in the relative degree of attraction to and repulsion from pheromones [133]. In addition, mutants in the pheromone-regulated *daf-7* TGF-β pathway have strong effects on aggregation. To determine whether pheromones are required for the elevated social behavior promoted by reduced *exp-1* activity, I quantified the social behavior of *exp-1(ox276) daf-22(ok693)* double mutant animals. The elevated social behavior of *exp-1(ox276)* animals was completely suppressed by the *daf-22(ok693)* mutation, indicating that pheromones are required for the high social behavior of *exp-1(ox276)* animals (**Figure 3.6**).

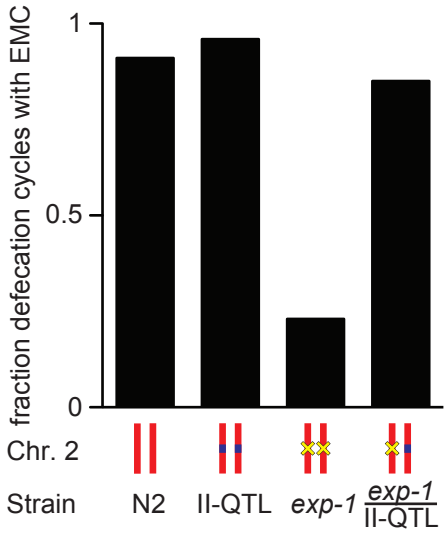

Figure 3.4

The HW II-QTL has full *exp-1* activity in the defecation program. Fraction of posterior body contractions (pBoc) followed by an enteric muscle contraction (EMC). The *ox276* deletion allele of *exp-1* was used.

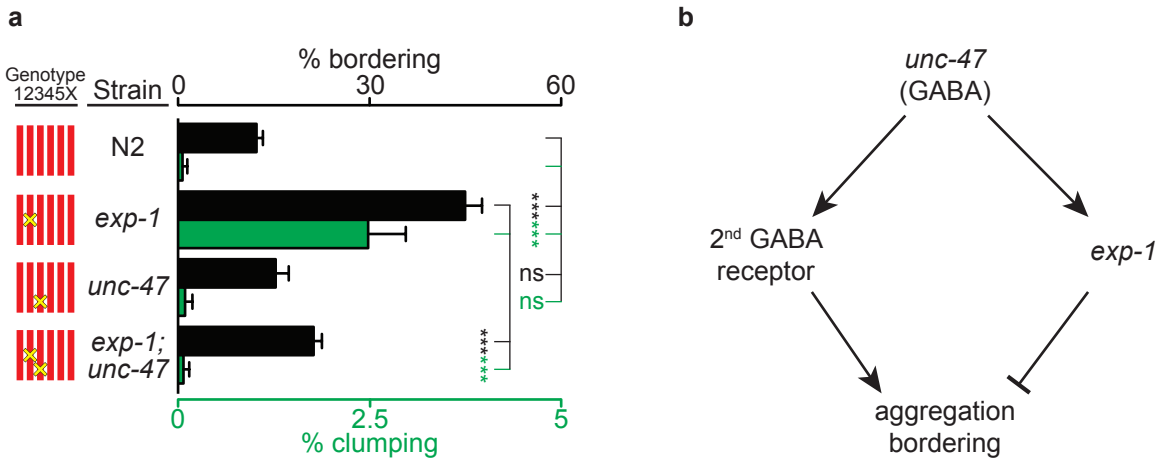

Figure 3.5

***exp-1* mutant animals require the vesicular GABA transporter *unc-47* for their increased social behavior.** **a)** Bordering and clumping behaviors of *exp-1(ox276)*, *unc-47(e307)*, and *exp-1(ox276);unc-47(e307)* mutant animals. Error bars, s.e.m. *** $P < 0.001$ by ANOVA with Bonferroni tests. **b)** Genetic model of GABA function in social behavior.

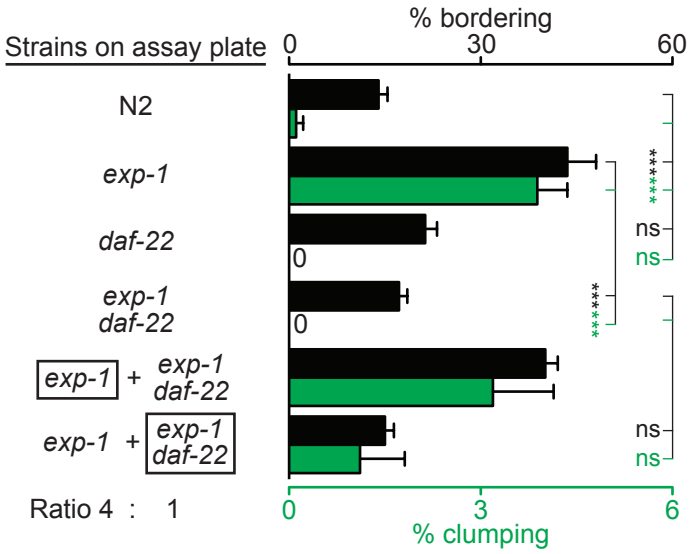

Figure 3.6

Pheromones are required for the elevated social behavior of *exp-1* mutant animals. Bordering and clumping behaviors of *exp-1(ox276)*, *daf-22(ok693)*, and *exp-1(ox276) daf-22(ok693)* mutant animals. *exp-1(ox276) daf-22(ok693)* were also assayed in the presence of *exp-1(ox276)* single mutants (bottom two pairs of bars); boxes indicate the strain whose behavior was measured. Error bars, s.e.m. *** $P < 0.001$ by ANOVA with Bonferroni tests.

daf-22 mutations affect the pheromones an animal produces; in a homogeneous culture, they also affect the pheromones it experiences, both in development and in the context of an acute aggregation assay. To test for acute effects of pheromones in the aggregation assay, I mixed *exp-1(ox276)* animals with *exp-1(ox276) daf-22(ok693)* animals marked with GFP in a 4:1 ratio and measured their social behavior. *exp-1(ox276) daf-22(ok693)* that could not produce pheromones retained their solitary behavior in the presence of *exp-1(ox276)* animals that did produce pheromones and aggregated (**Figure 3.6**). This result suggests that pheromones may not be an acute local signal driving aggregation of *exp-1(ox276)* animals; rather, pheromones may act during development to promote *exp-1(ox276)* social behavior, or pheromones may promote social behavior internally in each animal. Also, as *daf-22* mutants accumulate lipids that cannot be converted to pheromones [198], excess lipids in *daf-22(ok693)* may inhibit social behavior.

Bordering and clumping of *exp-1* mutants requires O_2-sensing neurons

Oxygen, and the oxygen-sensing neurons URX, AQR, and PQR, strongly modulate the social behavior of animals with reduced *npr-1* activity: aggregation is suppressed by a shift to low (~10%) O_2, by neuronal ablations or mutations affecting these neurons [199,168,200], and by decreasing the activity of these neurons by transgenic manipulations [201]. I next asked how these neurons affect the social behavior of animals with reduced *exp-1* activity. Killing URX, AQR, and PQR

suppressed the aggregation of animals with the HW II-QTL that reduces *exp-1* activity (**Figure 3.7**). Killing URX alone in animals with the HW II-QTL also trended towards reduced social behavior, but the effect was not as strong as killing URX, AQR, and PQR together (**Figure 3.7**). Interestingly, while killing URX, AQR, and PQR together in N2 does not modify their social behavior (data not shown), killing URX alone increased their bordering behavior (**Figure 3.7**). This result suggests that URX normally inhibits bordering behavior in N2, and that killing AQR and PQR in addition to URX reverses the increased social behavior induced by killing URX.

The BAG neuron senses downshifts in O_2 and changes in CO_2 levels [176,177,202]. BAG's role in social behavior, however, had not been previously examined. Killing BAG enhanced the bordering and clumping behaviors of the HW II-QTL and the bordering of N2 (**Figure 3.7**), suggesting that BAG normally inhibits social behavior.

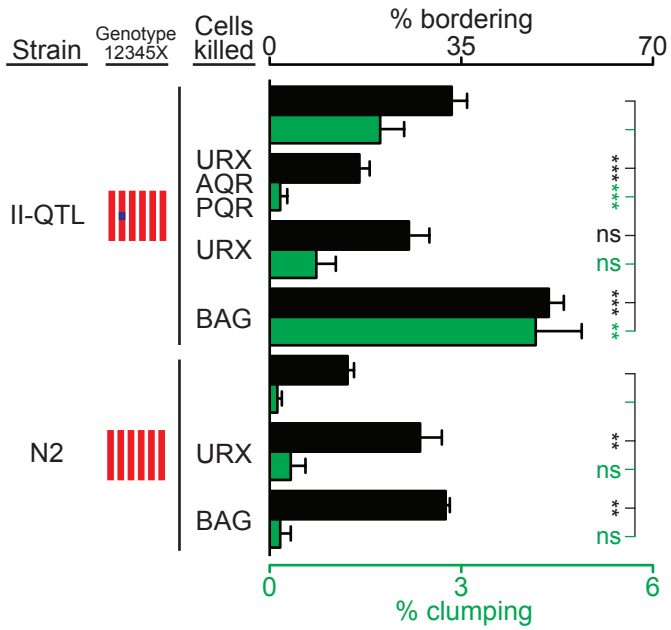

Figure 3.7

Social behavior consequences of killing O_2-sensing neurons. Bordering and clumping behaviors after killing URX, AQR, and PQR together, URX alone, or BAG alone, in II-QTL and N2 animals. Error bars, s.e.m. ** $P < 0.01$, *** $P < 0.001$ by ANOVA with Bonferroni tests.

Discussion

Using a combination of quantitative genetic techniques, this work identified a novel QTL that modulates social behavior in *C. elegans*. Fine-mapping of the QTL by screening a large number of recombinants narrowed down the location of the QTL to a 6.2 kb interval that contains 11 polymorphisms. Quantitative complementation tests and loss-of-function experiments were consistent with noncoding variation in the QTL affecting the GABA receptor *exp-1*. Further confirmation that *exp-1* is the gene affected by the QTL should come from gain-of-function experiments that rescue the low-activity social QTL with transgenes containing the high-activity solitary allele of *exp-1*.

exp-1 is the third gene identified that contributes to social behavior differences between wild-type *C. elegans* strains. The other genes are *glb-5,* which affects the sensation of O_2 and CO_2 cues that promote aggregation [62], and *npr-1,* which acts in the RMG neuron that integrates multiple aggregation-promoting stimuli [61,133]. Other QTL remain to be discovered, as *npr-1*, *glb-5*, and *exp-1* do not account for all bordering and aggregation behavior differences between wild-type strains. Strategies to identify these additional QTL are discussed in Chapter 4.

My data show that the *exp-1* social behavior QTL requires the activity of the URX, AQR, and PQR O_2-sensing neurons to promote social behavior, consistent with the role of O_2 in promoting aggregation behavior. Interestingly, while killing URX, AQR, and PQR neurons together suppresses *exp-1* social

behavior, killing URX alone does not strongly affect *exp-1* social behavior suggesting that these three neurons act redundantly or that AQR and PQR are more relevant than URX for this behavior. Killing each of these neurons individually and in combinations should help establish the contribution of each neuron. My experiments also revealed a previously unrecognized role of the URX and BAG O_2-sensing neurons in maintaining solitary behavior in N2 animals.

exp-1 is the only known GABA-gated channel that transports cations and thus functions as an excitatory GABA receptor. Previously, *exp-1* was known for its excitatory action on enteric muscles during defecation, but *exp-1* expression in *C. elegans* neurons unrelated to defecation suggested that it could have other roles as well [194]. My results are consistent with dual roles of GABA in social behavior: GABA inhibits social behavior through *exp-1*, but may promote it through additional receptors (**Figure 3.5b**). Genetic and functional studies, as well as sequence homology searches, have identified 8 GABA receptor genes in the *C. elegans* genome: 4 $GABA_A$, *exp-1*, an *exp-1*-related receptor, and 2 $GABA_B$ receptors. Combining mutations in other GABA receptor genes with an *exp-1* mutation can be a useful approach to identify GABA receptors that promote social behavior: mutations in GABA receptors that promote social behavior should suppress the increased social behavior of *exp-1* mutants. Identifying the cells where *exp-1* acts to affect social behavior will also be an important step toward understanding its function.

My results indicate that pheromones are necessary for *exp-1* social behavior. Pheromones are an important mode of communication between members of a species. In mammals and insects pheromones regulate mating, aggression, aggregation, and strain and sex recognition, among other behaviors [203-205]. In *C. elegans*, pheromones released by hermaphrodites attract males and are differentially attractive to hermaphrodites depending on their *npr-1* allele [192,193,133]. Strains with a social allele of *npr-1* find low concentrations of pheromones attractive, but strains with a solitary allele of *npr-1* find pheromones repulsive at all concentrations [133]. Nevertheless, animals with a social *npr-1* allele aggregate strongly even in the presence of a *daf-22* mutation that eliminates production of many short-chain pheromones [206]. The high social behavior of *exp-1* mutants, by contrast, requires *daf-22*, suggesting that pheromones are an important component of *exp-1* social behavior. Interestingly, low-activity *exp-1* animals do not use pheromones as an acute signal to aggregate with other animals. One possibility is that pheromones modify neuronal circuits for social behavior during *C. elegans* development, or during chronic exposure in adult animals. Pheromones regulate chemoreceptor gene expression in ASI, ASH, and AWC sensory neurons, illustrating how pheromones can create lasting changes in the *C. elegans* nervous system [207,208]. Indeed, pheromone exposure during development affects olfactory plasticity of adult *C. elegans*, setting a precedent for developmental effects of pheromones on adult

behavior [209]. It will be interesting to determine how pheromone exposure allows *exp-1* animals to become more social as adults.

Two parallel pathways that regulate social behavior have been identified in *C. elegans*, the neuropeptide receptor *npr-1* pathway and the *daf-7* TGF-β pathway [210,206]. *daf-7* transcription in the ASI pheromone-sensing neurons is negatively regulated by pheromones [211,212], providing a plausible link between pheromone sensation and aggregation behavior. The importance of pheromones for *exp-1* social behavior suggests that *exp-1* may be part of the *daf-7* pathway. Epistasis analyses of *exp-1;npr-1* and *exp-1;daf-7* double mutants should help determine whether *exp-1* is part of the *npr-1* pathway, the *daf-7* pathway, or a third novel social behavior pathway.

"Sometimes you are too clever to understand men, especially when they act almost as simply as animals."

–G.K. Chesterton

CHAPTER 4: Discussion and future directions

My thesis work focused on discovering the genetic basis of behavioral diversity between individuals of a species and on understanding how genetic variability affects the neuronal circuits that generate behavior.

Neuronal circuits for exploratory behavior

In Chapter 2 I described the identification of a genetic variant that affects the decision to abandon depleting resources and explore the environment. This variation results from polymorphisms that affect mRNA levels of the *tyra-3* gene, which encodes a receptor for the modulatory amine tyramine. High levels of *tyra-3* in sensory neurons that detect food cues promote exploitation of current resources, whereas lower levels promote exploration of the environment.

A better understanding of the circuits that affect exploration–exploitation decisions in *C. elegans* should come from identifying the cellular sources of tyramine. Initial experiments suggest that non-neuronal sources of tyramine may be important (see Appendix 2). Cell-specific rescue should identify the source of tyramine involved in exploratory behavior. Higher temporal resolution is required

to understand the timing of tyramine release that regulates exploratory behavior. Genetic tools that report the activation of a receptor upon ligand binding, such as the TANGO system developed for flies and mammalian cells, would help in this regard [213]. To this end, I have started to introduce the TANGO system into *C. elegans*.

My work also suggests that additional receptors for tyramine and the related monoamine octopamine are involved in exploratory behavior (Appendix 2); characterizing these additional receptors will further our understanding of this behavior.

How *tyra-3* affects sensory neurons that detect food cues is still unclear. Genetic experiments coupled to cell ablations indicate that *tyra-3* reduces the activity of ASK neurons that detect attractive amino acids, while it activates the BAG neurons that sense O_2 and CO_2 changes that may be associated with bacterial metabolism. Initial functional imaging of these neurons using the genetically-encoded calcium indicator GCaMP 3.0 failed to detect differences in activity of these neurons (data not shown). This negative result suggests that *tyra-3* may modulate aspects of neuronal physiology that are not reflected in overall calcium levels. Other monoamines, including octopamine (which is structurally and functionally related to tyramine), are known to selectively modulate specific routes of neuronal output, such as increasing the strength of electrical synapses while decreasing the strength of chemical synapses [214,215]. Similarly, *tyra-3* may modify specific aspects of synaptic function, such as the

release of classical neurotransmitters or neuropeptides, which may require different synaptic components for their release [216,217].

As shown in **Figure 2.4a**, I detected effects of genetic variation in *tyra-3* on exploratory behavior only in a HW genetic background, but not in an N2 background. The HW loci that are required by *tyra-3* to affect exploration have not been identified (see Appendix 2); characterizing them would further our understanding of natural variation in exploratory behavior in general and of the role of *tyra-3* in particular. One approach to identify the remaining HW loci is to measure the behavior of additional recombinant inbred advanced intercross lines (RIAILs). The N2-HW RIAILs panel consists of 236 strains, and I only measured the exploratory behavior of 91 of them. Testing more lines would increase the power to detect additional QTL by simple interval mapping and by searching for QTL that interact epistatically with the *tyra-3* QTL. As described in more detail in Appendix 2, one of the HW loci required by *tyra-3* to affect exploration may be *glb-5*. Combining HW alleles of *glb-5* and *tyra-3* in an N2 background would reveal if *glb-5* is indeed one of the HW loci needed by *tyra-3.*

The GABA system in social behavior

In Chapter 3 I describe the identification of a novel social behavior QTL in *C. elegans*. Genetic complementation and loss-of-function experiments are consistent with *exp-1* being the gene affected by the QTL. Further confirmation should come from gain-of-function experiments that rescue the low-activity social

QTL with transgenes containing the high-activity solitary allele of *exp-1*. It will be interesting to determine whether additional genes are affected by the QTL to modify to social behavior. Quantitative complementation with other genes in the region should identify these additional genes.

Together with *npr-1* and *glb-5*, *exp-1* is the third identified gene that modulates social behavior between wild-type *C. elegans* strains. Whereas coding changes in *npr-1* and *glb-5* impact social behavior, noncoding polymorphisms located 2-8 kilobases 3' of *exp-1* affect *exp-1* activity, modulating social behavior. Since these polymorphisms are not located in *exp-1* coding regions, they likely affect *exp-1* transcriptional regulation. Therefore, the magnitude, timing, or cellular sites of *exp-1* expression may vary between social and solitary animals. Characterizing these differences will be a topic of future research.

Other social behavior QTLs remain to be discovered, as *npr-1*, *glb-5*, and *exp-1* do not account for all bordering and aggregation behavioral differences between wild-type strains. Some of these additional QTL are probably in chromosomes V (where *glb-5* is located) and X (where *npr-1* is located): in an N2 genetic background, a HW chromosome V leads to higher social behavior than a strain carrying a small HW region that includes *glb-5*; the same is true for chromosome X and *npr-1*. The location of the additional chromosome V social behavior QTL is suggested by the QTL results shown in **Figure 3.1d**, where two closely linked peaks (one of which contains *glb-5*) cross the significance

threshold. To test whether this second peak represents an independent social behavior QTL, it would be useful to construct a near-isogenic line that carries HW DNA encompassing the second peak in an N2 background.

QTL mapping with the RIAILs did not show any significant peaks on chromosome X, but the existence of social QTL in this chromosome is inferred from the higher social behavior of a full HW X chromosome in an N2 background compared to a HW *npr-1* near-isogenic line. Since I was interested in identifying novel social QTL, I only used RIAILs that had the N2 solitary *npr-1* allele. As a consequence of testing only RIAILs with N2 *npr-1*, regions closely linked to *npr-1* are more likely to be of N2 origin, so few of the RIAILs carry HW loci in these positions, decreasing the power to detect QTL near *npr-1*. Therefore, one possibility is that the additional QTLs are located near *npr-1*. I, along with others in the Bargmann lab, have created near-isogenic lines of HW segments near *npr-1* in an N2 background. It would be useful to screen these strains for the presence of additional social behavior QTL. Alternatively, a panel of near-isogenic lines that covers the entire HW X chromosome in an N2 background [47] can be tested for social behavior.

While the genetic and neuronal analyses of *exp-1* in social behavior are still in their early stages, several interesting observations have already surfaced. For example, the role of the GABA system in *C. elegans* social behavior had not previously been recognized. Moreover, my work supports the idea that

pheromones are involved in *C. elegans* aggregation, and revealed that their effects may be chronic rather than acute.

Ongoing experiments are focused on understanding how genetic variation in *exp-1* modifies neuronal circuits that regulate social behavior. A first step will be to identify the neurons where *exp-1* acts. Finding the GABAergic neurons that activate *exp-1* will also be important. The *C. elegans* nervous system contains 26 GABAergic neurons, comprising six neuronal classes. Promoters that drive expression in subsets of these classes are known, and they can be used to rescue aggregation in *unc-47* GABA deficient mutants to find the relevant source of GABA. My work also suggests that while *exp-1* acts to inhibit bordering and aggregation behaviors, other GABA receptors promote these behaviors. Identifying these additional receptors and the neurons in which they act will further illuminate the neuronal circuits that generate *C. elegans* social behavior.

Pheromones do not appear to be an acute signal that promotes aggregation, but may be required by *exp-1* animals chronically or during development. To test this idea, *exp-1 daf-22* double mutant animals that cannot synthesize short-chain pheromones can be grown in the presence of purified pheromones and then tested for social behavior as adults in the absence of pheromones. Additionally, *exp-1 daf-22* animals can be grown in the presence of animals that do synthesize pheromones and then tested as adults. More refined experiments can determine whether specific pheromones are required, and whether they act at specific developmental stages.

Noncoding polymorphisms are commonly observed in behavioral variation

A feature of biological systems that facilitates evolvability – the degree to which organisms can evolve through adaptive changes – is the modularity of system components [218]. Modular components can change without disrupting the whole system, and these changes occasionally increase fitness. One type of modularity in biological systems is the structure of *cis*-regulatory elements (CREs) that regulate gene expression. The transcription of many genes is regulated by multiple independent CREs and this independence facilitates evolution by changing gene expression in certain tissues without disrupting expression in others [219].

Variation in the CREs that regulate transcription is the main type of genetic change driving morphological evolution [219]. For example, variation in abdominal pigmentation and trichome patterns between *Drosophila* species is caused by polymorphisms in CREs that affect *tan* and *shavenbaby* transcription, respectively [220,221]. The genetic changes underlying behavioral variation between and within species are only beginning to emerge, but available data suggest that noncoding changes affecting transcriptional regulation will also predominate. In mice, variation in noncoding sequences of *Rgs2* modulates anxiety traits between wild-type strains [79]. In voles, changes in expression patterns of the vasopressin receptor V1a affect pair-bonding behavior [139]. In different fly strains, expression levels of *Cyp6a20* correlate with aggressive behavior and changes in *for* expression affect foraging behavior [72,67]. In *C. elegans*, noncoding changes in

tyra-3 modulate exploratory behavior [63] and noncoding variation in *exp-1* affects social behavior.

Interestingly, the only two coding changes that emerged from QTL studies of *C. elegans* behavior, those affecting the *npr-1* and *glb-5* genes, arose during laboratory domestication. This domestication process involved a drastic change from a natural environment in association with rotting fruits to a laboratory environment with radically different properties: a single dominant food source, *E. coli,* and high levels of exposure to environmental O_2, along with many other changes that we have yet to appreciate [62].

Neurotransmitter receptors as a source of genetic behavioral variation

My results add to a growing body of evidence that genetic variation affecting neurotransmitter receptors is a frequent source of behavioral variation. In *C. elegans,* variation in the neuropeptide receptor *npr*-1 and in the GABA receptor *exp-1* affect social behavior, and polymorphisms in the tyramine receptor *tyra-3* modify exploratory behavior. In different vole species, variation in the brain expression pattern of the vasopressin receptor V1a affect pair-bonding behavior [139]. In humans, variation in serotonin, dopamine, acetylcholine, and several neuropeptide receptors has been suggested to affect different aspects of behavior [146,148,222,149,147]. Most of these neurotransmitter receptors, like *npr-1* and *tyra-3*, are G-protein coupled receptors. However, *exp-1* and the nicotinic acetylcholine receptors that modulate substance-abuse risk in humans are ligand

gated ion channels, suggesting that neurotransmitter receptors in general can be genetic sources of behavioral diversity.

By analogy to the facilitation of evolution by modular transcriptional regulation units, modularity in neurotransmission has the potential to be relevant to the evolution of behavior. A single neuron can respond both to multiple neurotransmitters and to the same neurotransmitter through multiple receptors. In this sense, some neurotransmitter receptors are modular components that can change without disrupting whole nervous systems.

Summary

The contribution of genetics to behavioral variation in humans and other animals has long been recognized, but the identity of the specific genes involved remained elusive. Using a combination of quantitative genetics tools and neuronal analyses I identified genetic variation in two neurotransmitter receptors as a source of behavioral diversity. One of these receptors uses information about the internal state of the animal to modify the activity of sensory neurons, while the other receptor is part of the GABA system that regulates social behavior. The neurobiological characterization of these receptors and the study of the mechanism by which genetic variation modifies neuronal circuits further our knowledge of the biological bases of behavior.

APPENDIX 1

The nature of behavior, and challenges in the study of behavior

Behaviors can be divided along different lines based on their complexity or their purpose. A classical ethological approach might classify behaviors based on whether they are related to food acquisition, predator avoidance, habitat selection, reproduction, or other social interactions (see Table below). A neurobiological approach might classify behaviors based on the extent to which they are learned, or based on the anatomical brain systems involved; this analysis would be orthogonal to the ethological one.

	Simple component	Complex pattern or sequence
Feeding behavior	Eating	Food selection and preference
Defensive behavior	Escape	Behavioral suppression, hiding
Habitat selection	Chemo/thermotaxis	Nest building, exploration
Reproductive behavior	Mating	Courtship song or dance
Other social behavior	Aggregation	Territorial defense, migration

A typical behavior involves a set of connected actions that take place over a period of time – for example, immediate withdrawal of a paw from a hot surface; rapid retreat from the area; licking the paw; long term-avoidance of the area. A sequence of behavioral actions is rarely as stereotyped as suggested by

this example, however, and even simple animals show a range of actions and sequences during a behavior like escape, mating, or grooming. To capture this variability, over the past decade the analysis of animal behavior has become more sophisticated with the increasing use of high-throughput, automated systems for behavioral monitoring, combined with statistical analysis of behavioral events[223-225]. These tools are particularly useful for genetic analysis, where quantitative data must be gathered for many individuals. Careful and accurate behavioral measurements are key to the genetic analysis of behavior.

The special challenge to understanding behavior is that the external and internal variables that affect behavior change over time, and a common response is only expected when all variables are held constant. As a result, gene–environment interactions are prominent features of behavioral variation. Variation is a property of many biological systems, but an animal's morphology is much more stable over time than its behavior. Variation is essential to behavior, not peripheral.

Although behavioral variation is conceptually interesting, it generates challenges both intellectually and technically. Natural behavioral variation exists on a continuum – genetic variants change the probability of certain behaviors in a quantitative way, not a qualitative way. Complicating the issue, behavioral measurements in mice differ significantly between laboratories even when care is taken to standardize every aspect of an experiment[226]. Even within a laboratory, anxiety measurements in mice are strongly influenced by the person handling the

animals[227]. Moreover, even when all variables are held constant, the behavioral response is often probabilistic rather than deterministic, which may have adaptive value[228]. With these factors in mind, it is clear why it is an art to develop behavioral assays that are both specific and sensitive.

APPENDIX 2

Potential Autosomal QTLs Affecting Leaving Behavior

An analysis of chromosome substitution strains (CSS) with a single HW chromosome in an N2 background confirmed the importance of the X chromosome but failed to show individual effects of the five HW autosomes on leaving behavior (**Figure A2.1a**). The CSS strains were generously provided by Man-Wah Tan (Stanford University). These results suggest that any autosomal QTLs are likely to be subject to epistatic interactions with each other or loci on X.

The initial RIAIL analysis identified a QTL on chromosome II, *leav-3*, in addition to the QTL on X (**Figure 2.1d**). However, HW chromosome II from the chromosome substitution strain did not have a significant effect on leaving either on its own (**Figure A2.1a**), or in combination with the HW region on X spanning *npr-1* and *tyra-3* (**Figure A2.1b**). This result suggested that mapping the QTL on II would be sensitive to additional epistatic interactions, and therefore the QTL was not pursued further.

A separate analysis of the RIAIL leaving data revealed a potential QTL on chromosome IV. This analysis was stimulated by the idea that animals near the border might be expected to leave more often than animals far from the border. The alternative leaving probability was calculated as the number of leaving events divided by the time that animals spent on the lawn, within 1 mm of the border. This analysis yielded two QTLs: the X chromosome QTL, and a QTL on

chromosome IV, *leav-4*, that just reached genome-wide significance (**Figure A2.1c**). HW chromosome IV from the chromosome substitution strain did not have a significant effect on leaving either on its own (**Figure A2.1a**), in combination with the HW region on X spanning *npr-1* and *tyra-3*, or when a full HW II chromosome was present (**Figure A2.1b**) so this QTL was not pursued further.

The leaving behavior of a strain containing HW chromosomes II and IV in combination with the QTL on X containing *npr-1* and *tyra-3*, is indistinguishable from a strain containing the X chromosome QTL alone (**Figure A2.1b**). This suggests that additional loci that were not detected by QTL mapping are required for the high leaving rate of HW animals. One of these additional loci could be *glb-5*, a functional gene in HW but nonfunctional in N2 due to a partial gene duplication that creates an early stop codon [62,106]. Isaac Strong and I found that *glb-5* modulates leaving behavior in a different format of the leaving assay – HW *glb-5* promotes leaving a very thin bacterial lawn (10-fold lower bacterial density than the lawn in the standard leaving assay used in the experiments in Chapter 2) when high density bacterial lawns are also present at the edges of the assay plate (data not shown). Combining HW *glb-5* with HW chromosomes II and IV, and the HW X chromosome QTL could reveal if a HW *glb-5* allele is required by the chromosomes II and IV QTLs to increase the leaving rate in an N2 genetic background.

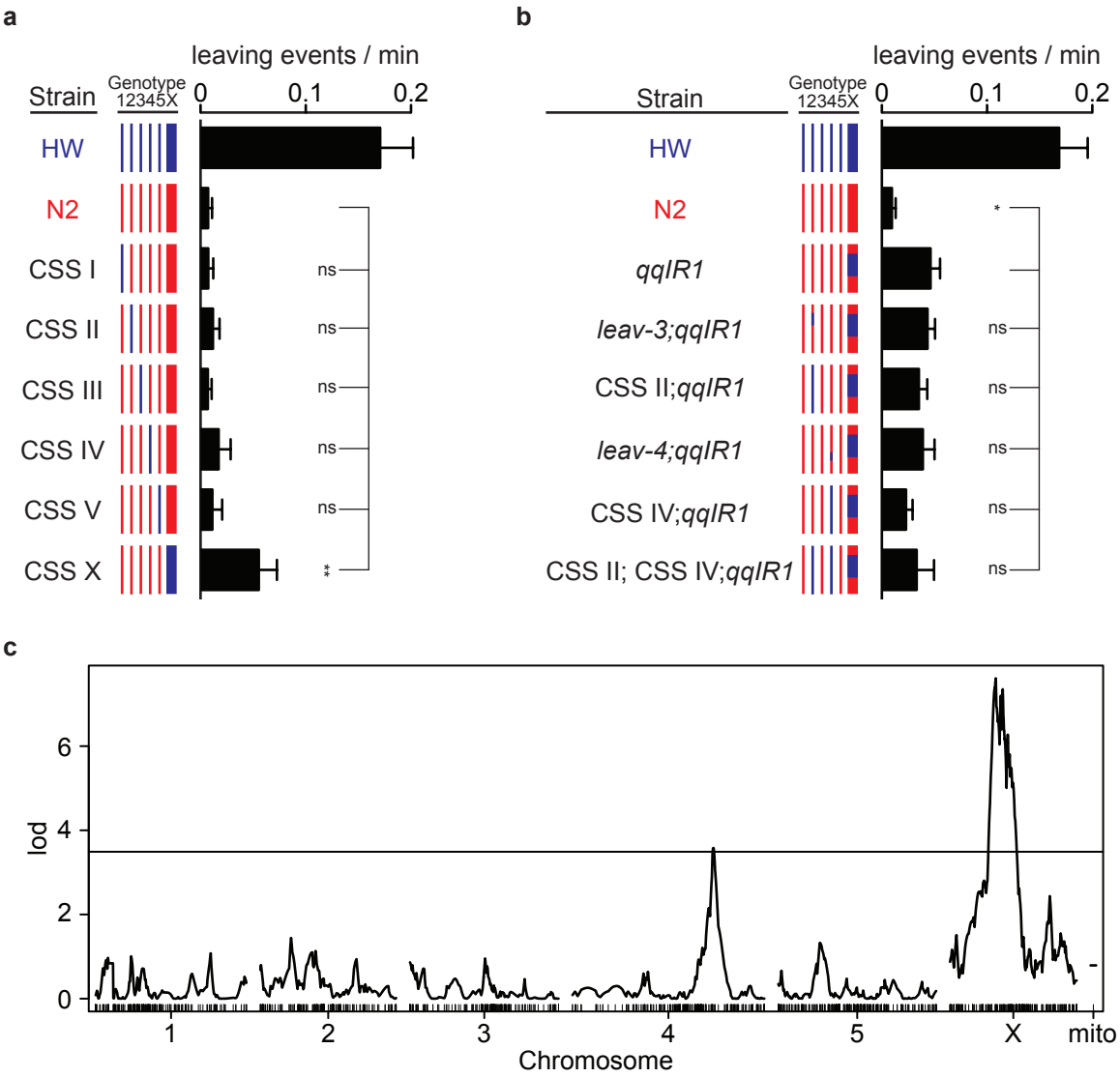

Figure A2.1

Exploration of autosomal QTLs involved in leaving behavior. a) Leaving rates in chromosome substitution strains that replace each N2 chromosome with a HW chromosome. **b)** Leaving rates in NILs containing autosomal QTLs in combination with the X chromosome QTL, in an N2 background. *leav-3* and *leav-4* correspond to the QTLs identified in Figure 2.1d and in panel **c** of this figure, respectively. **c)** Alternative QTL analysis of RIAILs for leaving events normalized not to total time on the lawn, but to time spent within 1 mm of the border of the lawn. The horizontal line denotes the $P < 0.01$ genome-wide significance threshold. Error bars indicate s.e.m. ** $P < 0.01$ by ANOVA with Dunnett test to correct for multiple comparisons.

Genetic interactions between *tyra-3, npr-1,* and genetic background

The octanol avoidance assay revealed a startling diversity of genetic interactions between *tyra-3, npr-1,* and genetic background. The N2 and HW *tyra-3* polymorphisms only affected octanol avoidance in the N2 background, not in the HW background (**Figure A2.2**). By contrast, the *tyra-3* polymorphisms only affected the leaving assay in the HW genetic background (**Figure 2.4a**). The N2 *npr-1* allele enhanced octanol avoidance in both N2 and HW backgrounds (**Figure A2.2**). In the N2 background, *tyra-3* and *npr-1* polymorphisms had a synergistic interaction: the HW *tyra-3* allele enhanced avoidance, the HW *npr-1* allele diminished avoidance, and the combination of HW *tyra-3* and HW *npr-1* diminished avoidance more than HW *npr-1* alone. Thus HW *npr-1* reversed the sign of the HW *tyra-3* effect. Further confirmation that these effects are mediated by *npr-1* and *tyra-3* and not by other genes in the introgressed regions may be obtained by transgenic rescue of *npr-1* and *tyra-3*.

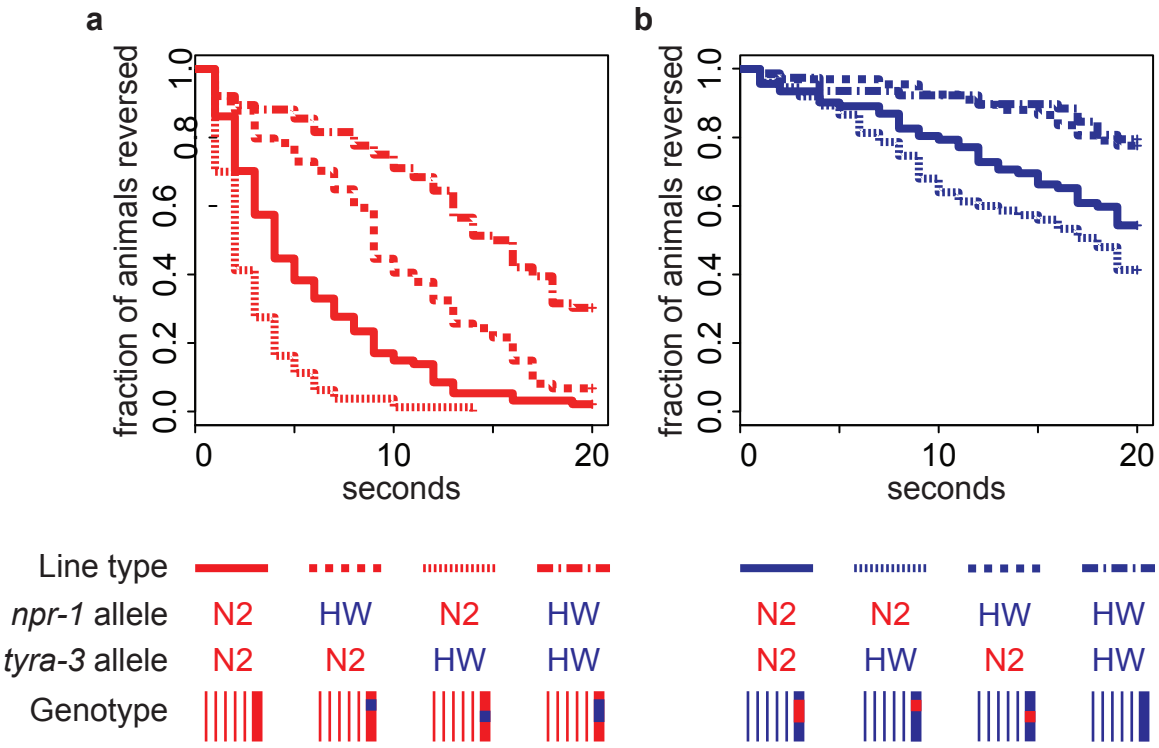

Figure A2.2

Interactions between *npr-1*, *tyra-3*, and genetic background for octanol avoidance. Kaplan-Meier curves representing the fraction of animals that have reversed after exposure to octanol. **a)** Strains in an N2 genetic background. **b)** Strains in a HW genetic background.

Role of tyramine and octopamine in leaving behavior

The experiments addressing the role of *tyra-3* in leaving behavior suggest that tyramine sensed by *tyra-3* reduces the tendency to leave a patch of food (**Figure 2.4**). The high-activity N2 allele of *tyra-3* is associated with a lower leaving rate than the low-activity HW allele (**Figure 2.4a**). Consistent with this notion, the reduction of the leaving rate induced by transgenic overexpression of *tyra-3* positively correlates with the transgene concentration (**Figure 2.4b**). Moreover, reducing the activity of *tyra-3* through RNAi also reduces the leaving rate (**Figure 2.4c**).

To test the roles of tyramine and octopamine in leaving behavior more directly I measured the leaving behavior of animals bearing mutations in the enzymes that synthesize these monoamines. Tyramine is produced from tyrosine through decarboxylation by *tyrosine decarboxylase 1 (tdc-1)*, while octopamine is produced by the hydroxylation of tyramine by *tyramine β-hydroxylase 1 (tbh-1)* (**Figure A2.3a**) [178]. If tyramine prevents leaving a patch of food, an animal that is unable to synthesize tyramine should have an increased leaving rate. Instead, HW animals with a *tdc-1* mutation, which lack tyramine and octopamine, had a reduced leaving rate, suggesting that tyramine or octopamine or both prevent leaving through *tyra-3*, but promote leaving through alternative receptors (**Figure A2.3b**). HW animals with a *tbh-1* mutation that impairs their production of octopamine had an elevated leaving rate compared to HW, suggesting that octopamine prevents leaving (**Figure A2.3b**). Animals with a

tbh-1 mutation, however, not only lack octopamine but also accumulate tyramine at 20-fold higher levels than wild type animals [178]. Therefore, the increased leaving rate of *tbh-1* mutants could result from increased tyraminergic signaling and not from reduced octopamine, a result consistent with the reduced leaving rate of *tdc-1* mutant animals that lack tyramine. Additional experiments are needed to separate the roles of tyramine and octopamine in leaving behavior and identify the additional receptors for these monoamines that mediate their actions.

tdc-1, which produces tyramine, is expressed in two neuron pairs, RIM and RIC, and in two non-neuronal tissues, the gonadal sheath, which is part of the somatic gonad and uterine cells UV1 [178]. *tbh-1*, which converts tyramine into octopamine, is expressed in the RIC neurons and in the gonadal sheath. Initial attempts to identify the source of tyramine and octopamine relevant to leaving behavior consisted in cell-specific rescue of *tdc-1* of HW animals with a *tdc-1* mutation. HW animals with a *tdc-1* mutation have a reduced leaving rate. Restoring *tdc-1* to the RIM neurons or to the RIC neurons plus the gonadal sheath transgenically with cell-specific promoters driving *tdc-1* cDNA did not rescue the reduced leaving rate of HW animals with a *tdc-1* mutation (**Figure A2.3c**). The *tdc-1* cDNA is functional, since it can rescue the social behavior phenotypes of *tdc-1* mutants animals shown in **Figure 2.14** (data not shown). Therefore, the results suggest that tyramine and octopamine from these individual neuronal and nonneuronal sources is insufficient to affect leaving behavior. If a single cell is important, these results point to the UV1 cells – which

were the only cells in which rescue was not performed – as the relevant source of tyramine for leaving behavior. Cell-specific rescue in the UV1 cells will be required to confirm this hypothesis. Initial attempts at finding a good promoter for the UV1 cells have been unsuccessful: I was unable to obtain reliable GFP expression in the UV1 cells using an *egl-38* promoter reported to drive expression in the these cells [229].

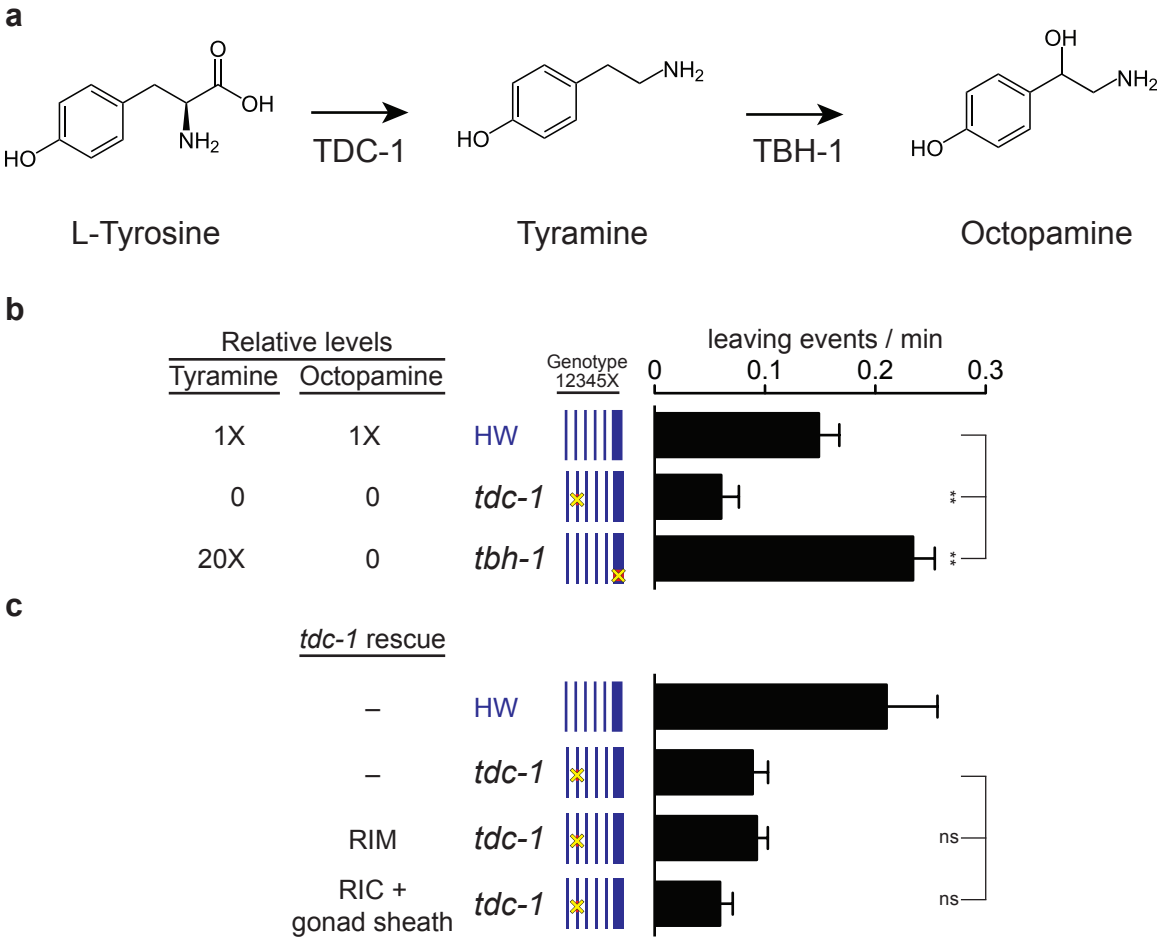

Figure A2.3

Effect on the leaving rate of mutations that affect tyramine and octopamine synthesis. a) Tyramine and octopamine synthesis pathway. TDC-1, tyrosine decarboxylase 1. TBH-1, tyramine β-hydroxylase. **b)** Leaving behavior of HW animals and HW animals with *tdc-1(n3420)* and *tbh-1(n3247)* mutations. *tdc-1(n3420)* and *tbh-1*(n3247) were introgressed into HW from an N2 background. Relative monoamine levels were measured in an N2 background [178]. **c)** Cell-specific rescue of *tdc-1*. The *gcy-13* promoter was used for RIM, and the *tbh-1* promoter for RIC and the gonadal sheath. Error bars indicate s.e.m. ** $P <$ 0.01 by ANOVA with Dunnett test.

METHODS

Nematode Growth

Strains were grown and maintained under standard conditions at 22-23 ˚C on nematode growth medium (NGM) 2% agar plates [230]. All animals used for behavioral assays were grown on plates seeded with dense *E. coli* HB101 lawns.

Analysis of Behavior in the Leaving Assay

6 cm NGM agar plates were seeded with 70 μL (conditioning plate) or with 10 μL (assay plate) of a fresh overnight culture of *E. coli* HB101 diluted in LB to OD_{600nm}=2.0. 90 min after seeding the plates, ten young adult hermaphrodites were picked onto the conditioning plate. 30 min after being placed on the conditioning plates, seven of the animals were transferred onto the lawn of the assay plate. The 30 min leaving assay began 1 hr after placing the seven animals on the assay plate. The number of leaving events was recorded manually by examining the video recordings, and further behavioral analysis was conducted with a Matlab code adapted from the Parallel Worm Tracker with the help of Makoto Tsunozaki [231]. A leaving event was defined as an episode in which the whole body of an animal left the bacterial lawn and the animal did not reverse immediately to return to the lawn. The leaving rate was calculated as the number of leaving events per worm minute spent inside the bacterial lawn. Experiments on each strain were repeated at least three times.

Quantitative Trait Locus Analysis

The N2-HW recombinant inbred advanced intercross lines (RIAILs) used in this study represent the terminal generation of a 20-generation pedigree founded by reciprocal crosses between N2 and HW. The lines were constructed through 10 generations of intercrossing followed by 10 generations of selfing [43]. They have been genotyped at 1454 nuclear and one mitochondrial markers and have a 5.3-fold expansion of the F2 genetic map [43]. QTL analysis was performed on the mean leaving rates, bordering, and clumping of N2-HW RIALs by nonparametric interval mapping in R/qtl [232]. Significance levels were estimated from 10,000 permutations of the data.

Identification of the Minimal Genetic Region for the *leav-2* QTL

The genetic region from 4.78-5.75 MB defined by the *leav-2* strain in **Figure 2.4** encompassed 158 genes. The location of the potential leaving-suppressing variant was inferred from the analysis of breakpoints within the QTL in individual RIAILs, as follows. The 1.5-lod score confidence interval defined by the RIAILs spans from ~4.6 to 5.3 Mb (**Figure 2.5**). Thus, the causal variant in *leav-2* was hypothesized to lie between 4.78 and 5.3 Mb, a region containing only 85 genes. Six RIAILs that had HW *npr-1* had a breakpoint to the left of 5.75 Mb. Three of the strains (QX108, QX122, and QX202) had N2 sequence to the right of 4.93 Mb and low leaving rates (**Figure 2.5**). The other three (QX75, QX154, QX158) had N2 sequence to the right of 5.03 Mb and higher leaving rates.

These observations suggested that the N2 allele that suppresses leaving may lie between 4.93 and 5.03 Mb, a region that contains 17 genes (**Figure 2.6**).

Identification of *tyra-3* as the Gene Affected by the *leav-2* QTL

PCR products that overlap by at least 1 kb, covering the region from 4.93 to 5.03 Mb on the X chromosome, were amplified from N2 genomic DNA using the following primers and injected into HW animals (at 5 ng/μL) in groups:

Product a (*tyra-3*).Forward cctgctcttttctggaggtg, reverse gccgcaaaaacagagaaaac

Product b. Forward ttttccttttagatctccatgtc, reverse tgaaggaaccgtattttccaa

Product c. Forward ttttccttttagatctccatgtc, reverse aaagcggatcaagaattcca

Product d. Forward ccaccatgtacccaggaatc, reverse ccttcctcgagtcaagttgc

Product e. Forward agaacaaccccgagacacac, reverse tggagttttccaccgatttc

Product f. Forward ccaatcacctgccctttcta, reverse tgtggacgatgagttggtgt

Product g. Forward cgactcaaaggtgcaagaca, reverse gaagttcggctgaaaagcac

Product h. Forward aacctttcagccaccgtatg, reverse acgcgttcaagcacttttct

Product i. Forward gcaatttccatcctcatcgt, reverse ttcaacttccagtcggaacc

Product j. Forward gtgctcacaaaatcgcagaa, reverse gctcgagacattttcgaagg

Product k. Forward cgacaatgatggacacaagg, reverse agaagccgaagaaggaggac

Product l. Forward aacaaaattggctcgtgacc, reverse aacttttgttcccggatgtg

DNA pools tested by injection were pool 1=a+b; 2,=a; 3,=b; 4=c; 5=d; 6=e+f+g+h; 7=h+i+j+k+l. Three transgenic lines were tested per DNA pool.

Generation of Near-Isogenic Lines

Near-isogenic lines were created by backcrossing a chromosomal region or allele into the desired genetic background as described below. Desired segments were then inbred to homozygosity. For introgressions into the HW (CB4856) background, crosses were set up to avoid problems with the incompatibility locus between N2 and HW on chromosome I [233].

Marker positions are based on Wormbase release WS219; *npr-1* is at 4,769,595 (indel) and *tyra-3* is at 4,948,658 (indel).

QX1092 *npr-1 (CB4856>N2) [qqIR3]* X: QX202, a RIAIL containing CB4856 *npr-1* and N2 *tyra-3*, was crossed to *lon-2* males (in an N2 background) for 10 generations, picking non-Lon hermaphrodites each generation (*lon-2* is tightly linked to *npr-1).* The introgression breakpoints are, on the left, between 3,921,083 (marker haw101674) and 4,060,839 (marker pkP6146), and on the right, between 4,892,213 (marker pkP6106) and 4,937,279 (marker haw102792). There is additional CB4856 sequence with a left breakpoint between 6,073,091 (marker haw103987) and 6,278,584 (indel) and a right breakpoint between 6,278,584 (indel) and 6,581,237 (marker pkP6154).

CX11400 *leav-1 (N2>CB4856) [kyIR9]* X: CB4856 males were crossed to QX9, a RIAIL containing N2 *npr-1*, and a recombinant F2 between *npr-1* and an indel marker at 4,948,658 was selected. This recombinant bearing N2 *npr-1* and CB4856 sequence to its right was backcrossed to CB4856 males nine more times, selecting hermaphrodites with an N2 allele of *npr-1* each generation. The introgression breakpoints are, on the left, between 4,649,200 (marker uCE6-872)

and 4,745,912 (marker snp_C39E6[1]), and on the right, between 4,768,758 (marker snp_C39E6[4]) and 4,797,631 (marker uCE6-877).

CX10927 *leav-2 (N2>CB4856) [kyIR2]* X: QX122, a RIAIL containing CB4856 *npr-1* and N2 sequence to its right, was crossed to CB4856 males for 10 generations, selecting hermaphrodites with an N2 allele at 4,948,658 (indel) each generation. The introgression breakpoints are, on the left, between 4,769,595 (indel) and 4,797,631 (marker uCE6-877), and on the right, between 5,744,794 (indel) and 5,759,074 (marker uCE6-952).

CX13272 *npr-1 tyra-3 (N2>CB4856) [kyIR91]* X: QX32, a RIAIL containing N2 *npr-1* and *tyra-3*, was crossed to CB4856 males. Male F1s were backcrossed to CB4856 hermaphrodites, F2s were selfed, and F3s that kept N2 *npr-1* and *tyra-3* were crossed to CB4856 males again. This cycle was repeated four more times. The introgression breakpoints are, on the left, between 4,060,839 (marker pkP6146) and 4,279,605 (marker uCE6-854), and on the right, between 5,153,187 (marker uCE6-890) and 5,234,763 (marker uCE6-904).

CX11950 *tyra-3 (ok325>CB4856) [kyIR25]* X: CB4856 males were crossed to CX11839 (*tyra-3* [*ok325*]) and a recombinant F2 between *npr-1* and *tyra-3 (ok325)* was identified. This recombinant bearing CB4856 *npr-1* and *tyra-3 (ok325)* was backcrossed to CB4856 males eight more times, selecting hermaphrodites with an *ok325* allele of *tyra-3* each generation. The introgression breakpoints are, on the left, between 4,919,592 (marker haw102765) and 4,919,769 (marker haw102766), and on the right, between 5,360,624 (indel) and 5,414,461 (marker uCE6-929).

CX13271 *tyra-3 (CB4856>N2) [kyIR90]* X: QX75, a RIAIL containing CB4856 *npr-1* and *tyra-3,* was backcrossed repeatedly to N2-derived strains, while recombining *dpy-3* and *lon-2* markers on and off the X chromosome to eliminate linked sequences from CB4856. The introgression breakpoints are, on the left, between 4,866,708 (indel) and 4,919,592 (marker haw102765), and on the right, between 5,033,445 (marker uCE6-886) and 5,152,492 (marker uCE6-888).

QX1157 *npr-1 tyra-3 (CB4856>N2) [qqIR2]* X: QX125, a RIAIL containing CB4856 *npr-1* and *tyra-3*, was backcrossed to *lon-2* males (in an N2 background) for 20 generations, picking non-Lon hermaphrodites each generation. The introgression breakpoints are, on the left, between 4,507,511 (marker uCE6-865) and 4,637,513 (marker pkP6149), and on the right, between 10,265,260 (indel) and 11,142,289 (marker pkP6114).

CX14180 *npr-1 (CB4856>N2) [kyIR122]* X: QX1155, a near-isogenic line containing CB4856 *npr-1* in an N2 background that had been backcrossed 20 times to N2, was crossed to N2 and a recombinant that kept CB4856 *npr-1* but lost CB4856 DNA to the left of *npr-1* was isolated by Joshua Greene. The introgression breakpoints are, on the left, between 4,076,447 (indel) and 4,384,533 (indel), and on the right, between 4,769,595 (indel) and 4,892,213 (marker pkP6106).

CX11176-CX11177 *leav-3 (CB4856>N2) [kyIR4-kyIR5]* II: WE5237 (chromosome substitution strain II) males were mated to QX1157; male F1 were

mated to QX1157 and two F2 recombinants that kept CB4856 alleles at 1,683,956 (marker haw18527) and 5,329,512 (marker haw25319) were isolated.

CX11170 *leav-4 (CB4856>N2) [kyIR3]* IV: QX45 was backcrossed to *mec-3 dpy-4* (in an N2 background) for 10 generations, picking non-Mec, non-Dpy animals each generation. The introgression breakpoints are, on the left, between 11,508,357 (marker haw59342) and 11,603,955 (marker haw59377), and on the right, between 13,629,791 (marker haw61017) and 13,706,656 (marker haw61106).

CX13602 *II-QTL (CB4856>N2) [kyIR97]* II: QX111, a RIAIL containing the II-QTL was backcrossed to *clr-1 dpy-10* (in an N2 background) for 9 generations, picking non-Clr, non-Dpy animals each generation. A smaller introgressed region was further isolated through recombination with N2. The introgression breakpoints are, on the left, between 5,926,596 (indel) and 5,941,581 (indel), and on the right, between 6,195,603 (marker haw25802) and 6,198,696 (marker haw25803).

Fine-mapping of the social behavior II-QTL

N2 animals were crossed to *II-QTL (kyIR97*), F1 hermaphrodites were selfed and 5,000 individual F2 hermaphrodites were dispensed into individual wells of 96-well plates with the use of a worm sorter (COPAS Biosort system; Union Biometrica). These F2s were grown on 200 µL of an *E. coli* OP50 suspension in S-basal buffer with cholesterol, rotating at 230 RPM, at 22 ºC for 6 days. The progeny of F2s was genotyped at 5,941,581 (indel) and 6,195,603 (marker haw25802), a 0.41 cM interval, and recombinants between these

markers were isolated. The recombinant chromosome was then made homozygous. These homozygous recombinants were then tested for social behavior.

tyra-3 Population Genetic Summary Statistics

Population genetic summary statistics for the gapless sites in the alignment of the *tyra-3* genomic region were calculated using *libseq* [234] by Matthew Rockman. SNP variation (17644.3 noncoding and synonymous sites, $p = 10.53 \times 10^{-4}$, $Q_w = 9.99 \times 10^{-4}$) is typical for *C. elegans* genes [235,236]. The allele frequency spectrum is also consistent with neutral equilibrium (Tajima's $D = 0.368$, $p = 0.6$). The SNP data provided no evidence for intragenic recombination ($R_{min} = 0$).

Quantitative RT-PCR

Total RNA from mixed stage worms was isolated with Trizol. 1.5 mg of RNA and oligo-dT were used for reverse transcription using SuperScript III First-Strand Synthesis (Invitrogen) according to the manufacturer's instructions. Real-time PCR was performed with Fast SYBR Green Master Mix (Applied Biosystems) on a 7900HT Real-Time PCR System (Applied Biosystems). *act-3* was used as the calibrator for relative quantitation. 5' primers corresponded to upstream exons that distinguished *tyra-3* isoforms, and 3' primers corresponded to shared exon sequence. Primers used were:

tyra-3a&c.2_F, ccacttgcaaatagcagcag

tyra-3b_F, ggctatttggtggtggtttg

tyra-3a & tyra-3b_R, tccttctggcgtcgaaatac

act-3_F, tcacgatcatgagaccattcaaa

act-3_R, gcaaattgtagtggggtcttcttatg

tyra-3 Expression Pattern

The N2 and HW 4.9 kb *tyra-3b* promoters were amplified using primers: tcaacctaaccactaactaaggg and cGatgaagcaagatgtcaggt, which overlaps the coding region by 4 bp. The ATG start codon is mutated to ATC (mutation is uppercase in primer). These promoters were individually fused by PCR to a fragment containing GFP followed by the *unc-54* 3'-UTR, as described [237]. These PCR products were injected individually into both HW and N2 animals at 20 ng/μL. Cells expressing GFP were identified by Nomarski microscopy in both L1 and adult hermaphrodites. The identification of some cells was aided by injecting *Ptyra-3b*::GFP-expressing animals with promoter-mCherry fusions with established expression patterns. In this manner, the AIM neurons were identified as *Ptyra-3b*::GFP-expressing cells based on their position and the absence of co-localization with *Pttx-3*::mCherry. The BAG neurons co-expressed *Ptyra-3b*::GFP and *Pflp-17*::mCherry. The CEP neurons co-expressed *Ptyra-3b*::GFP and *Pdat-1*::mCherry. The ASK neurons co-expressed *Ptyra-3b*::GFP and *Psra-*

9::mCherry. The ADL neurons co-expressed *Ptyra-3b*::GFP and *Psri-51*::mCherry.

Extrachromosomal transgenes

Transgenes were made by injection of DNA clones into the gonads of young adult hermaphrodites together with a fluorescent coinjection marker [238]. To control for variation between transgenes, between two and five independent lines from each injection were characterized.

RNAi

RNA interference was performed essentially as described [239]. A fragment common to all isoforms of a gene was amplified. The following primers were used, which include the T7 sequence (underlined):

*tyra-3*_F, taatacgactcactatagggagagaaaatggcagcaggacttt

*tyra-3*_R, taatacgactcactatagggagaatcctcgcagtctgtggagt

*exp-1*_F, taatacgactcactatagggagacatgggtttcattatggatgg

*exp-1*_R, taatacgactcactatagggagacaatgaaatcggtgcattgt

*abts-3*_F, taatacgactcactatagggagatgaaagcagctaggacagca

*abts-3*_R, taatacgactcactatagggagagcgtacgatccagtgaatga

in vitro transcription was performed with RiboMAX kit (Promega). dsRNA was injected at 1.2 mg/mL into the gonads of adult hermaphrodites. Eggs laid 24 and 48 hours after injection were used for the behavioral assays.

Generation of MosSCI Lines and Quantitation of GFP Fluorescence in ASK

Single-copy insertion of transgenes was performed using the direct MosSCI technique targeting the *ttTi5605* Mos allele on chromosome II, as described [172]. A schematic of the mechanism underlying MosSCI is shown in Figure 2.10c.

The pCFJ151 targeting vector was modified by the introduction of an FseI restriction site into the multiple cloning site by site-directed mutagenesis using the primers gtaatacgactcacttaaggccggccctagagggtaccagagctcacc and ggtgagctctggtaccctctagggccggccttaagtgagtcgtattac to make pAB1. An FseI-SpeI fragment from a pSM vector containing *N2-Ptyra-3b::N2-tyra-3b::SL2 GFP::unc-54 3'-UTR* or *HW-Ptyra-3b::N2-tyra-3b::SL2 GFP::unc-54 3'-UTR* was cloned into pAB1.

For each *tyra-3*-containing test plasmid, about fifty EG4322 animals were injected with a mixture of *tyra-3* plasmid, pGH8, pCFJ90, pCFJ104, and pJL43.1. After positive and negative selection and full sequencing of the insert, two inserted transgenes each of N2-*Ptyra-3b* and HW-*Ptyra-3b* were backcrossed to HW males seven times, selecting GFP-fluorescent hermaphrodites each generation. The transgene-containing chromosome was then homozygosed.

The strains containing the single-copy transgene in a HW background were injected with *Psra-9*::mCherry to identify ASK. Young adult hermaphrodites were examined on a Zeiss Imager Z.1 with a 60X objective focused on ASK using mCherry to prevent bleaching of GFP signal. Fluorescence signals were

acquired with fixed acquisition times (30-50 msec for mCherry, 100 msec for GFP). Background mean fluorescence intensity adjacent to ASK was subtracted from the ASK signal.

Octanol Avoidance Assay

Avoidance assays were conducted essentially as described [240]. In brief, ~20 three-day old animals were picked off of their growth plates food into a transfer plate without bacteria where they were allowed to crawl and rid themselves of bacteria. Animals were then transferred onto an NGM plate without food. After 40 minutes, a microcapillary with 30% octanol (v/v diluted fresh every day in ethanol) was presented in front of the animal's nose. The time to reverse was recorded. If animals did not reverse within 20 seconds, the assay was stopped. Animals were presented with odor 1-3 times per experiment, with at least 3 minutes of rest interval. I replicated published results demonstrating that *tyra-3* null mutants had more rapid responses than N2 in the presence of exogenous serotonin and tyramine [122] but also observed more rapid responses in the absence of exogenous neuromodulators, as shown in Figure 2.7.

Cell Ablations

For leaving behavior assays ASK was ablated with a laser microbeam as described [241]. BAG and URX were killed using split human caspase 3 fragments [242] expressed from *flp-17* and *glb-5* promoters that overlapped only in BAG or *flp-*

8 and *glb-5* promoters that overlapped only in URX. These strains were made by Patrick McGrath. URX, AQR, and PQR were killed jointly using egl-1 cell death activator driven by the *gcy-36* promoter [168]. For lysine chemotaxis assays, ASK was killed using a mouse caspase 1 gene expressed from the *sra-9* promoter [243]. The ASK strain was a generous gift from Ryuzo Shingai.

Social Behavior Assay

Aggregation and bordering behaviors were measured essentially as described [61], with the modifications from [133]. Briefly, 2-3 week old (kept at 4 ºC) 2% agar NGM plates were seeded with 200 μL of a saturated *E. coli* OP50 bacterial culture in LB 2 days before the assay. 150 animals adult animals were picked onto the assay lawn. Bordering and clumping behavior were quantified after two hours at 22 ºC. An animal was considered to be bordering if its whole body resided within 1 mm of the border of the bacterial lawn. Clumping behavior was measured as the fraction of animals that were in contact with two or more other animals along at least 50% of their body length.

Defecation Assay

A defecation cycle consists of a posterior body contraction (pBoc), followed by an anterior body contraction (aBoc), and concludes with an enteric muscle contraction (EMC). Defecation assays were performed as described [194].

I scored for the number of pBoc that were followed by an EMC. At least 25 animals per strain were scored. Each animal was scored only once.

Transgenes

The *Pflp-21::LoxP stop LoxP::npr-1 SL2 GFP* and *Pncs-1::nCre* constructs are described in Macosko et al, 2009 [133].

The 11 kb *tyra-3* genomic transgene was amplified using Expand Long Range dNTPack (Roche) with primers cctgctcttttctggaggtg and gccgcaaaaacagagaaaac

Ptyra-3b was amplified using primers: tcaacctaaccactaactaaggg and cGatgaagcaagatgtcaggt, which overlaps the coding region by 4 bp. The ATG start codon is mutated to ATC (mutation is uppercase in primer). This product was cloned into pSM.

ADL Psri-51 ends: gactgtaaaatcgataagca…ccactgccaccgggcagaac

ASK Psra-9 ends: gcatgctatattccaccaaa...tgtgcatcaatcatagaaca

BAG Pflp-17 ends: ccttgaagcttttcctctga…gcaaaactttatttttccag

CEP, ADE, PDE Pdat-1 ends: atctctgaaatgtttctagt…aatctcaacaatttttagcc

tyra-3b cDNA was cloned by RT-PCR. The ends are atggctatttggtggtggtt… agcaatcgacaatattctaa. The product was cloned into pSM with NheI and KpnI.

Promoter-GFP fusions were performed as described [244], with the same promoter end sequences as in *Ptyra-3b*.

The 12.6 kb *tyra-3* genomic transgene was cloned into pSM. The ends are agttggtacaaaaagcttac…gttctcagggtgattgtgtt. The 184 bp deletion was engineered by site-directed mutagenesis.

Strains

'Wild-type' strains

Strain	Origin	Haplotype [43]
N2	Bristol, England	1
CB4856	Hawaii, USA	41
MY1	Lingen, Germany	29
MY14	Mecklenbeck, Germany	40
JU258	Madeira, Portugal	39
CB4853	Altadena, California, USA	19

N2-HW RIAILs for leaving QTL analysis

QX10, QX11, QX12, QX13, QX14, QX24, QX31, QX32, QX34, QX37, QX38, QX39, QX42, QX43, QX45, QX47, QX49, QX52, QX55, QX56, QX57, QX58, QX61, QX62, QX64, QX65, QX68, QX70, QX71, QX73, QX74, QX75, QX77, QX82, QX86, QX91, QX98, QX107, QX108, QX121, QX122, QX124, QX125, QX126, QX127, QX128, QX129, QX131, QX132, QX133, QX134, QX144, QX149, QX151, QX152, QX154, QX157, QX158, QX165, QX167, QX168, QX169, QX171, QX174, QX175, QX176, QX178, QX179, QX181, QX182, QX185, QX187, QX190, QX191, QX192, QX193, QX195, QX196, QX198,

QX199, QX200, QX202, QX205, QX208, QX209, QX210, QX212, QX213, QX221, QX223, QX233

N2-HW RIAILs for bordering and clumping QTL analyses

QX1, QX3, QX4, QX5, QX6, QX8, QX9, QX15, QX16, QX17, QX18, QX20, QX25, QX26, QX27, QX29, QX32, QX33, QX38, QX43, QX44, QX47, QX48, QX49, QX51, QX52, QX53, QX54, QX55, QX56, QX57, QX62, QX68, QX70, QX71, QX72, QX73, QX74, QX76, QX78, QX79, QX80, QX81, QX82, QX83, QX84, QX85, QX87, QX90, QX92, QX93, QX94, QX95, QX96, QX97, QX99, QX100, QX102, QX103, QX110, QX112, QX114, QX115, QX120, QX121, QX128, QX129, QX137, QX140, QX147, QX156, QX157, QX161, QX163, QX165, QX171, QX174, QX175, QX176, QX177, QX178, QX181, QX186, QX187, QX189, QX190, QX192, QX193, QX194, QX203, QX206, QX210, QX212, QX216, QX217, QX218, QX220, QX221, QX227, QX228, QX230, QX231.

Near-isogenic lines in a HW genetic background:

CX11400 *kyIR9 [leav-1 X:~4.70-~4.78Mb, N2>CB4856]*

CX10927 *kyIR2 [leav-2 X:~4.78-~5.75Mb, N2>CB4856]*

CX13272 *kyIR91 [X:~4.17-~5.19Mb, N2>CB4856]*

CX11950 *kyIR25 [tyra-3 (ok325) X:~4.92-~5.39Mb, CX11839>CB4856]*

Near-isogenic lines in an N2 background:

QX1092 *qqIR3 [X: ~3.99-~4.91Mb, CB4856>N2]*

CX13271 *kyIR90 [X:~4.89-~5.09Mb, CB4856>N2]*

QX1157 *qqIR2 [X: ~4.57-~10.70Mb, CB4856>N2]*

CX14180 *kyIR122 [X: ~4.24-~4.83Mb, CB4856>N2]*

CX11176-CX11177 *kyIR4-kyIR5 [II: ~1.68-~5.33Mb, WE2537>N2]; qqIR2*

CX11441 *kyIR11 [II CB4856>N2]; qqIR2*

CX11170 *kyIR3 [IV: ~11.6-~13.6Mb, CB4856>N2]; qqIR2*

CX11442 *kyIR12 [IV: CB4856>N2]; qqIR2*

CX13602 *kyIR97 [II: ~5.93-~6.97Mb, CB4856>N2]*

CX11498 *kyIR11 [II CB4856>N2]; kyIR12 [IV CB4856>N2]; qqIR2*

CX10774 *kyIR1 [V: glb-5, CB4856>N2]*

Chromosome substitution strains:

These strains carry a single CB4856 chromosome in an N2 background. They were a gift from Man-Wah Tan.

WE5236 *[I, CB4856>N2]*

WE5237 *[II, CB4856>N2]*

WE5238 *[III, CB4856>N2]*

WE5239 *[IV, CB4856>N2]*

WE5240 *[V, CB4856>N2]*

WE5241 *[X, CB4856>N2]*

Transgenic strains

CX12787, CX12788 *kyEx3586, kyEx3589 [Pflp-21::LoxP stop LoxP::npr-1 SL2 GFP @50ng/mL, Pelt-2::mCherry @2ng/mL]*

CX12789-CX12790 *kyEx3590-kyEx3591 [Pncs-1::nCre @20ng/mL, Pofm-1::dsRed @10ng/mL]*

CX10576, CX10577, CX10595, CX10596 *kyEx2635, kyEx2636, kyEx2651, kyEx2652 [11kb tyra-3 HW-genomic fragment @5ng/mL; Pelt-2::GFP @4.5ng/mL]*

CX10457-CX10459 *kyEx2536-kyEx2538 [11kb N2-tyra-3 genomic fragment @5ng/mL; Pelt-2::GFP @4.5ng/mL]*

CX10619-CX10621 *kyEx2667-kyEx2669 [11kb HW-tyra-3 genomic fragment @1ng/mL; Pelt-2::GFP @4.5ng/mL]*

CX10622-CX10624 *kyEx2670-kyEx2672 [11kb N2-tyra-3 genomic fragment @1ng/mL; Pelt-2::GFP @4.5g/mL]*

CX11362, CX11365 *kyEx3025, kyEx3028 [HW-Ptyra-3b::HW-tyra-3b::SL2 GFP @5ng/mL]*

CX11367-CX11368 *kyEx3030-kyEx3031 [HW-Ptyra-3b::N2-tyra-3b::SL2 GFP @5ng/mL]*

CX11363-CX11364 *kyEx3026-kyEx3027 [N2-Ptyra-3b::N2-tyra-3b::SL2 GFP @5ng/mL]*

CX11366, CX11369 *kyEx3029, kyEx3032 [N2-Ptyra-3b::HW-tyra-3b::SL2 GFP @5ng/mL]*

CX10790-CX10791 *kyEx2762-kyEx2763 [N2-Ptyra-3b::GFP @20ng/mL; Pelt-2::GFP @4.5ng/mL]*

CX10789, CX10792 *kyEx2761, kyEx2764 [HW-Ptyra-3b::GFP @20ng/µL; Pelt-2::GFP @4.5ng/mL]*

CX13452-CX13456 *kyEx4030-kyEx4034 [12.6kb N2-tyra-3 genomic fragment @5ng/mL; Pelt-2::GFP @4.5g/mL]*

CX13447-CX13451 *kyEx4025-kyEx4034 [12.6kb N2-tyra-3 genomic fragment Δ184 @5ng/mL; Pelt-2::GFP @4.5g/mL]*

CX13112-CX13114 *kyEx3778-kyEx3780 [Psri-51::N2-tyra-3b::SL2 GFP @50ng/µL, Pelt-2::mCherry @2ng/µL]*

CX11495-CX11497 *kyEx3063-kyEx3065 [Psra-9::N2-tyra-3b::SL2 GFP @40ng/mL, Pelt-2::GFP @4.5ng/mL]*

CX13118-CX13120 *kyEx3784-kyEx3786 [Pflp-17::N2-tyra-3b::SL2 GFP @1ng/µL, Pelt-2::mCherry @2ng/µL]*

CX13115-CX13117 *kyEx3781-kyEx3783 [Pdat-1::N2-tyra-3b::SL2 GFP @25ng/µL, Pelt-2::mCherry @2ng/µL]*

QS4 *qrIs2 (Psra-9::mCaspase 1, Pelt-2::GFP)*

CX13355 *kyIR92 [kyIs538 (Pglb-5::p12 hCaspase 3::SL2 GFP, Pelt-2::mCherry), CX11697>CB4856]; kyIR93 [kyIs536 (Pflp-17::p17 hCaspase 3::SL2 GFP, Pelt-2::GFP), CX11697>CB4856]*

CX11674-CX11675 *kyIR14-kyIR15 [kySi47,kySi46 (Cbr-unc-119(+)::N2-Ptyra-3b::N2-tyra-3b::SL2 GFP) II, N2>CB4856]*

CX11673, CX11676 *kyIR13,kyIR16 [kySi41,kySi43(Cbr-unc-119(+)::HW-Ptyra-3b::N2-tyra-3b::SL2 GFP) II, N2>CB4856]*

CX13972 *exp-1(ox276) daf-22(ok693) II; kyEx4313 [Pelt-2::GFP @4.5 ng/uL]*

CX11697 *kyIs536 [Pflp-17::p17 hCaspase 3::SL2 GFP, Pelt-2::GFP]; kyIs538 II [Pglb-5::p12 hCaspase 3::SL2 GFP, Pelt-2::mCherry]*

CX11728 *kyIs537 [Pflp-8::p17 hCaspase 3::SL2 GFP, Pelt-2::GFP]; kyIs538 II [Pglb-5::p12 hCaspase 3::SL2 GFP, Pelt-2::mCherry]*

CX13666 *kyIR97 kyIs538 [Pglb-5::p12 hCaspase 3::SL2 GFP, Pelt-2::mCherry] II; kyIs536 [Pflp-17::p17 hCaspase 3::SL2 GFP, Pelt-2::GFP]*

CX13850 *kyIR97 kyIs538 [Pglb-5::p12 hCaspase 3::SL2 GFP, Pelt-2::mCherry] II; kyIs537 [Pflp-8::p17 hCaspase 3::SL2 GFP, Pelt-2::GFP]*

CX7102 *qaIs2241 [Pgcy-36::egl-1, Pgcy-35::GFP, lin-25(+)] X*

Mutant strains

DA609 *npr-1(ad609) X*

CX11839 *tyra-3(ok325) X*, outcrossed 4X to N2

CX13840 *abts-3(ok368) II*, autosomes outcrossed 3X and X chromosome outcrossed completely to N2

CX13975 *exp-1(ox276) II*, outcrossed 6X to N2

CX13976 *unc-47(e307) III*, autosomes outcrossed 3X and X chromosome outcrossed completely to N2

CX14021 *exp-1(ox276) II; unc-47(e307) III*

CX13846 *daf-22(ok693) II*, autosomes outcrossed 5X and X chromosome outcrossed completely to N2

CX13847 *exp-1(ox276) daf-22(ok693) II*

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