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but enhanced by GTP-γS and AlF₄⁻ (Fig. 2, C and D), indicating that the cytoplasmic domains of β₃ and β₁ can directly interact with G₁₃ and that GTP enhances the interaction. The G₁₃-β₃ interaction was enhanced in platelets adherent to fibrinogen, and by thrombin, which stimulates GTP binding to G₁₃ via GPCR (Fig. 2E). Hence, the interaction is regulated by both integrin occupancy and GPCR signaling.

To map the β₁ binding site in G₁₃, we incubated cell lysates containing Flag-tagged wild type or truncation mutants of G₁₃ (Fig. S5) with GST-β₃CD beads. GST-β₃CD associated with wild-type G₁₃ and the G₁₃ 1 to 212 fragment containing α-helical region and switch region I (SRI), but not with the G₁₃ fragment containing residues 1 to 196 lacking SRI (Fig. 2F). Thus, SRI appears to be critical for β₁ binding. To further determine the importance of SRI, G₁₃-β₁ binding was assessed in the presence of a myristoylated synthetic peptide, Myr-LLARRPTKGHIEY (mSRI), corresponding to the SRI sequence of G₁₃ (197 to 209) (21, 22). The mSRI peptide, but not a myristoylated scrambled peptide, inhibited G₁₃ binding to β₁ (Fig. 2G), indicating that mSRI is an effective inhibitor of β₁-G₁₃ interaction. Therefore, we further examined whether mSRI might inhibit integrin signaling. Treatment of platelets with mSRI inhibited integrin-dependent phosphorylation of c-Src Tyr⁴¹⁶ and accelerated RhoA activation (Fig. 3A). The effect of mSRI is unlikely to result from its inhibitory effect on the binding of RhoGEFs to G₁₃ SRI because G₁₃ binding to RhoGEFs stimulates RhoA activation, which should be inhibited rather than promoted by mSRI (22). Thus, these data suggest that β₁-G₁₃ interaction mediates activation of c-Src and inhibition of RhoA. Furthermore, mSRI inhibited integrin-mediated platelet spreading (Fig. 3B), and this inhibitory effect was reversed by C3 toxin (which catalyzes at 1 min after exposure of platelets to thrombin, indicating the presence of a negative regulatory signal (Fig. 4, D and F). Furthermore, thrombin-stimulated activation of RhoA occurs during platelet shape change before substantial ligand binding to integrins (Fig. 4, C, D, and F). In contrast, after thrombin stimulation, β₁ binding to G₁₃ was diminished at 1 min when G₁₃-dependent activation of RhoA occurs, but increased after the occurrence of integrin-dependent platelet aggregation (Fig. 4, E and F). Thrombin-stimulated binding of G₁₃ to α₁β₃ and simultaneous RhoA inhibition both require ligand occupancy of α₁β₃ and are inhibited by the integrin inhibitor Arg-Gly-Asp-Ser (RGDS) (Fig. 4, D to F). Thus, our study demonstrates not only a function of integrin α₁β₃ as a noncanonical G₁₃-coupled receptor but also a new concept of G₁₃-dependent dynamic regulation of RhoA, in which G₁₃ mediates initial GPCR-induced RhoA activation and subsequent integrin-dependent RhoA inhibition (Fig. 4G). These findings are important for our understanding of how cells spread, retract, migrate, and proliferate, which is fundamental to development, cancer, immunity, wound healing, hemostasis, and thrombosis.

The function of G₁₃ in mediating the integrin-dependent inhibition of RhoA contrasts with the traditional role of G₁₃, which is to mediate GPCR-induced activation of RhoA. However, GPCR-mediated activation of RhoA is transient, peaking at 1 min after exposure of platelets to thrombin, indicating the presence of a negative regulatory signal (Fig. 4, D and F). Furthermore, thrombin-stimulated activation of RhoA occurs during platelet shape change before substantial ligand binding to integrins (Fig. 4, C, D, and F). In contrast, after thrombin stimulation, β₁ binding to G₁₃ was diminished at 1 min when G₁₃-dependent activation of RhoA occurs, but increased after the occurrence of integrin-dependent platelet aggregation (Fig. 4, E and F). Thrombin-stimulated binding of G₁₃ to α₁β₃ and simultaneous RhoA inhibition both require ligand occupancy of α₁β₃ and are inhibited by the integrin inhibitor Arg-Gly-Asp-Ser (RGDS) (Fig. 4, D to F). Thus, our study demonstrates not only a function of integrin α₁β₃ as a noncanonical G₁₃-coupled receptor but also a new concept of G₁₃-dependent dynamic regulation of RhoA, in which G₁₃ mediates initial GPCR-induced RhoA activation and subsequent integrin-dependent RhoA inhibition (Fig. 4G). These findings are important for our understanding of how cells spread, retract, migrate, and proliferate, which is fundamental to development, cancer, immunity, wound healing, hemostasis, and thrombosis.

References and Notes
15. V. Senyak et al., Cancer Res. 69, 262 (2009).
21. Single-letter abbreviations for amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
27. This work was supported by grants HL08264, HL062350, and HL068819 from the National Heart, Lung, and Blood Institute (X.D.) and GM061454 and GM074001 from the National Institute of General Medical Sciences (T.K.). We thank G. Nucifora for help with bone marrow transplantation and K. O’Brien and M. K. Delaney for proofreading.

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Figs. S1 to S6
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Functional and Evolutionary Insights from the Genomes of Three Parasitoid Nasonia Species

The Nasonia Genome Working Group†

We report here genome sequences and comparative analyses of three closely related parasitoid wasps: *Nasonia vitripennis*, *N. giraulti*, and *N. longicornis*. Parasitoids are important regulators of arthropod populations, including major agricultural pests and disease vectors, and *Nasonia* is an emerging genetic model, particularly for evolutionary and developmental genetics. Key findings include the identification of a functional DNA methylation tool kit; hymenopteran-specific genes including diverse venoms; lateral gene transfers among Pox viruses, *Wolbachia*, and *Nasonia*; and the rapid evolution of genes involved in nuclear-mitochondrial interactions that are implicated in speciation. Newly developed genome resources advance *Nasonia* for genetic research, accelerate mapping and cloning of quantitative trait loci, and will ultimately provide tools and knowledge for further increasing the utility of parasitoids as pest insect-control agents.

Parasitoid wasps are insects whose larvae parasitize various life stages of other arthropods (for example, insects, ticks, and mites). Female wasps sting, inject venom, and lay eggs on or in the host, where the developing off-spring consume and eventually kill it. Parasitoids
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are widely used in the biological control of insect pests, and they are very diverse, with estimates of over 600,000 species (1, 2). Nasonia is the second genus of Hymenoptera to have whole-genome sequencing, after Apis mellifera (Fig. 1), and Nasonia comprises four closely related parasitoid species: N. vitripennis, N. giraulti, N. longicornis, and N. oneida (3, 4). Nasonia are sexually tractable organisms with short generation time (~2 weeks), large family size, ease of laboratory rearing, and cross-fertile species. Like other hymenopterans, haploid males develop from unfertilized eggs, and diploid females develop from fertilized eggs. Cross-fertile species facilitate the mapping and cloning of genes that are involved in species differences. Haploid genetics assist efficient genotyping, mutational screening (5), and evaluation of gene interactions (epistasis) without the added complexity of genetic dominance. As a result, Nasonia are now emerging as genetic model organisms, particularly for complex trait analysis, developmental genetics, and evolutionary genetics (4).

We sequenced, assembled, annotated, and analyzed the genome of N. vitripennis from sixfold Sanger sequence genome coverage by using a highly inbred line of N. vitripennis (6). The draft genome assembly comprises 26,605 contigs [total length of 239.8 Mb, with half of the bases residing in contigs larger than 18.5 kb (N50), 40.6% guanine plus cytosine content (GC)]. Contigs were placed with mate-pair information into 6,181 scaffolds (total size 295 Mb, N50 = 709 kb). We assessed the N. vitripennis assembly for completeness and accuracy by comparing it with 19 finished bacterial artificial chromosome (BAC) sequences and 18,000 expressed sequence tags (ESTs). The genome assembly contained 98% of the BAC and 97% of the EST sequences, with an error rate of 5.9 × 10−7. Thus the assembly is a high-quality representation of both genomic and transcribed N. vitripennis sequences.

Highly inbred lines of the two sibling species N. giraulti and N. longicornis (Fig. 1B) were sequenced with onefold Sanger and 12-fold, 45-base pair (bp) Illumina genome coverage. Assembled by alignment to the N. vitripennis reference using stringent criteria (6), these reads cover 62% and 62.6% of the N. vitripennis assembly, and 84.7% and 86.3% of protein coding regions, respectively. These were used for genome comparisons and provided resources [for example, single nucleotide polymorphisms (SNPs) and microsatellites] for scaffold, gene, and quantitative trait loci (QTL) mapping. Sequence error rates for the N. giraulti alignment are estimated to be 3.8 × 10−3 for the entire alignment and 1.47 × 10−4 for coding sequences on the basis of comparison to three finished N. giraulti BACs (6). Sequences of 25 coding genes in both species perfectly matched their respective aligned sequences.

Normally, the intracellular bacteria Wolbachia prevent the formation of interspecies hybrids; however, antibiotically cured strains are cross-fertile (7). Hybrid crosses (Fig. 1C) (6) were used to map scaffolds and visible mutations onto the five chromosomes of Nasonia (Fig. 2). Several interspecies QTL have already been mapped using genetic/genomic resources, including wing size (8, 9), host preference (10), female mate preference (11), and in this study, sex-ratio control and male courtship (6). Linkage analysis has revealed that the genome-wide recombination rate in Nasonia is 1.4 to 1.5 centimorgans (cM/Mb), which is lower than that of honeybees (12, 13), and shows a 100-fold difference in rate between high- and low-recombination regions of the genome (Fig. 2) (6).

An official gene set (OGS v1.1) was generated from comparisons to A. mellifera, Tribolium castaneum, Drosophila melanogaster, Pediculus humanus, Daphnia pulex, and Homo sapiens [details are given in (6)]. Overall, Nasonia encodes a typical insect gene repertoire (Fig. 3) (6), of which 60% of genes have a human ortholog, 18% are arthropod-specific, and 24% appear to be hymenoptera-specific, showing high conservation between Nasonia and Apis and low conservation or absence in other taxa. An additional 12% are either Nasonia-specific or without clear orthology. Many (63%) single-copy orthologs shared between Nasonia and Apis occur in microsynteny blocks, which is similar to the amount of microsynteny blocks in Aedes aegypti/Anopheles gambiae and H. sapiens/Gallus gallus (14). Four hundred and forty-five orthologs between Nasonia and humans lack a candidate homolog in D. melanogaster (table S1), including the human transcription factors E2F7 and E2F8, which are involved in cell-cycle regulation. Further refinement of the gene set resulted in OGS v1.2 (15), which totals 17,279 genes, of which 74% have tiling microarray or EST support (6).

Nasonia is abundant in transposable elements (TEs) and other repetitive DNA (table S2 and fig. S1). This contrasts with a paucity of TEs in A. mellifera (16). TE diversity in Nasonia is 30% higher (2.9 TE types/Mb) than the next most diverse insect (Bombyx mori, 2.1 TE types/Mb), and is 10-fold higher than the average dipteran (6, 17). Nasonia also contains an unusual abundance of nuclear-mitochondrial insertions and a higher density of microsatellites (10.9 kb/Mb) than most other arthropod species (18, 19), suggesting that the accumulation of repetitive DNA is a feature of these insects.

The Nasonia genome encodes a full DNA methylation tool kit, including all three DNA cytosine-5-methyltransferase (Dnmt) types (Fig. 1A).
In vertebrates, Dnmt3 establishes DNA methylation patterns, Dnmt1 maintains these patterns, and Dnmt2 is involved in tRNA methylation (20). The Nasonia genome encodes three Dnmt1 genes, one Dnmt2, and one Dnmt3, in contrast with D. melanogaster, which has only Dnmt2. The presence of all three subfamilies in both Nasonia and Apis (Fig. 1) raises the question of whether methylation has similar regulatory functions in Hymenoptera as it does in vertebrates. DNA methylation is important in Apis caste development (21) and is suggested for Nasonia sex determination (22). Coding exons of both Nasonia and Apis show bimodal distributions in observed/expected CpG (Fig. S2) (6, 23), which is consistent with mutational biases due to DNA methylation of hyper- and hypomethylated genes. We confirmed methylated CpG dinucleotides in five examined N. vitripennis genes by bisulfite sequencing (fig. S3). These results suggest that epigenetic modifications by DNA methylation may be important in Hymenoptera. Nasonia also has the largest number of ankyrin (ANK) repeat-containing proteins (over 200) so far found in any insect (table S3) (6), suggesting a regulatory importance through protein-protein interactions (24).

Systemic RNA interference (RNAi) in Nasonia allows for gene expression knockdowns (4, 25). The Nasonia genome encodes homologs for the majority of genes implicated in small RNA processes (table S4). However, as in Tribolium and Apis, Nasonia lacks an RNA-dependent RNA polymerase (RdRp) ortholog, indicating a different systemic RNAi mechanism than in Caenorhabditis. Using various computational approaches (6), we identified 52 putative micro RNAs (miRNAs) with homologies to known miRNAs (26), nine that were previously unknown, and 11 additional Hymenoptera-specific miRNAs (table S5). Small RNA library sequencing confirmed 39 predicted and identified 59 additional miRNAs (table S6).

Nasonia shares a long germ-band mode of embryonic development with Drosophila, but exhibits significant differences in the genetic mechanisms involved (5, 27, 28) (see fig. S4). All major components of the dorso-ventral patterning system are present, with many Nasonia-specific gene duplications in the Toll pathway. Orthologs of vertebrate genes absent from the dorsal-ventral patterning system are identified in the Toll pathway (29). Nasonia has the largest number of yellow/mauve major royal jelly (yellow/MRP) genes that are linked to caste formation and sociality (29). Nasonia has the largest number of yellow/MAJP genes so far found in any insect, including an independent amplification of MRJP-like proteins (fig. S5) (6, 29). Although their function in Nasonia is unknown, these genes are expressed broadly in different tissues and life stages (table S7). The insect sex peptide/receptor system, which causes female re-mating refactoriness (30), is highly conserved in insects but is absent in Nasonia and Apis (table S8) (6). Instead, Nasonia males inhibit female re-mating behaviorally with a special “post-copulatory display” (31). Additional features analyzed (6) include those related to sex determination (fig. S6), pathogens and immunity (fig. S7), neuro-peptides (tables S9 and S10), cuticular proteins (table S11), xenobiotics (fig. S8), and diapause (table S12).

We investigated genome microevolution, including rapidly evolving genes that are potentially involved in species differences and speciation, by using the genomes of the three closely related Nasonia species. Synonymous divergence between N. vitripennis and its sibling species N. giraulti and N. longicornis is 0.031 ± 0.0002 SE and 0.030 ± 0.0002 SE, respectively, and between N. giraulti and N. longicornis is 0.014 ± 0.0001 SE (6), which is comparable to those among Drosophila sibling species (32). We compared the ratio of synonymous-to-nonsynonymous substitutions (dN/dS) between Nasonia species pairs with respect to gene ontology (GO) term categories, using genes with high-quality alignments and 1:1 orthologs between Nasonia and Drosophila. Nuclear genes that interact with mitochondria revealed significantly elevated dN/dS [by comparison of dN/dS distributions for each GO term to resampled distributions, see (6) and table S13], specifically those encoding mitochondrial ribosomes (P < 0.003 for all species pairs) and oxidative phosphorylation complex I (P < 0.03 for N. vitripennis/N. giraulti and N. vitripennis/N. longicornis) and complex V (P < 0.04 for all species pairs). This finding is consistent with the rapid evolutionary rate of Nasonia mitochondrial (33) and studies implicating nuclear-mitochondrial incompatibilities in F2 hybrid breakdown (7, 31). For example, reciprocal crosses between N. giraulti × N. vitripennis have identical F1 nuclear genotypes, but their mitochondrial haplotypes differ. Yet, micro-array hybridization (Fig. 2) of DNA from pooled surviving adult F2 haploid males shows distortion in the recovery of particular regions of the genome, which is dependent upon their mitochondrial haplotype (giraulti versus vitripennis). Because hybrid mortality is post-embryonic (7) and embryo ratios are Mendelian (33), these distortions reflect larval to adult mortality. In particular, F2 males with N. vitripennis alleles on the left arm of chro-

![Fig. 2. A high-resolution recombination map of the five Nasonia chromosomes is shown (6), with estimated gene density and locations of visible markers, landmark genes, and QTL. The hybridization percentage to N. vitripennis alleles is shown among surviving adult N. vitripennis × N. giraulti F2 hybrid males with either N. vitripennis (green curve) or N. giraulti (orange curve) mitochondria. Dots specify genome regions with significant differences in the hybridization ratio between the reciprocal crosses (P < 0.01).](https://www.sciencemag.org/)
mitochondria suffer nearly 100% mortality (Fig. 2). This region contains three 
genes encoding mitochondrial interacting proteins, atpD, ampK, and nadh-ubiquinone 
oxireductase (Fig. 2). Coevolution of nuclear and mitochondrial genomes can 
accelerate evolution (34, 35), and these findings indicate that such interactions 
contribute to reproductive incompatibility and speciation in Nasonia.

Sequences of 25 gene regions from multiple 
strains for the three Nasonia species (6) show low 
levels of intraspecific variation (table S14) with 
synonymous site variation ranging from 0.0005 
in N. giraulti to 0.0026 in N. vitripennis, which 
are much lower than in Drosophila species and 
more akin to levels observed in humans (36). 
This low nuclear variation could be explained by 
founder events, purging of deleterious mutations 
in haploid males, or inbreeding.

Recent lateral gene transfers from the 
bacterial endosymbiont Wolbachia into the genomes 
of Nasonia and other arthropods have been 
identified (37). Detecting ancient lateral transfers is 
more problematic. By examining protein domain 
arrangements in Nasonia relative to other organ-
isms, we uncovered an ancient lateral gene transfer 
involving Pox viruses, Wolbachia, and Nasonia. 
Thirteen ANK repeat–bearing proteins encoded in 
the N. vitripennis genome also contain C-terminal 
PRANC (Pox proteins repeats of ankyrin-
C terminal) domains. This domain was previously only de-
scribed in Pox viruses, where it is associated with 
ANK repeats and inhibits the nuclear factor kB 
(NF-kB) pathway in mammalian hosts (38). A 
computational screen revealed ANK-PRANC– 
bearing genes in some Wolbachia and a related 
Rickettsiales (Fig. 4). Screening additional Wol-
bachia confirmed the presence of ANK-PRANC 
genes in diverse Wolbachia. The Nasonia PRANC 
genes are clearly integrated in the genome (6) and 
are expressed in different life stages (table S15). 
Phylogenetic analysis of the PRANC-domain se-
quences suggests that the Nasonia lineage acquired 
one or more of these proteins from Wolbachia, 
with subsequent amplification and divergence (Fig. 4). 
Such lateral gene transfers between bacteria and 
animals could be an important source of evolu-
tionary innovation (37).

Nasonia is a carnivore, feeding on an amino 
acid–rich diet both as larva and adult (4). Mapping 
of Nasonia genes onto metabolic pathways 
(39) revealed loss or rearrangement in some amino 
acid metabolic pathways, including tryptophan 
and aminosugar metabolism (fig. S9) (6). The 
changes may reflect its specialized carnivorous 
diet and can inform efforts to produce artificial 
diets for more economical parasitoid rearing.

The venom of parasitoids, injected into a host 
before oviposition, serves to condition the host for 
successful development of wasp progeny (1, 2). 
Unlike the defensive Apis venom that inflicts pain 
and damage, parasitoid venoms have diverse phys-
iological effects on hosts, including developmen-
tal arrest; alteration in growth and physiology; 
suppression of immune responses; induction of 
paralysis, oncosis, or apoptosis; and alteration of 
host behavior (40). The identification of Nasonia
genes with venom features and proteomic analyses of venom reservoir tissues have uncovered a rich assemblage of 79 candidate venom proteins (table S16) (47). Some Nasonia venom reservoir proteins belong to previously known insect venom families such as serine proteases; however, nearly half were not related to any known insect venoms. As expected, many of these venom candidates show highly elevated expression in the female reproductive tract, which includes the venom glands and reservoirs. Venom genes also showed significantly higher dN/dS ratios between N. vitripennis and N. giraulti than nonvenom genes did (Mann-Whitney U test, P < 2 × 10−5), suggesting that changes in host use between the species may be accompanied by rapid evolution of venom proteins. The large venom protein set found in Nasonia with diverse physiological effects (40) and abundance of parasitoid species (1, 2) suggests that parasitoids may contain a rich venom pharmacopeia of potential new drugs.

N. vitripennis is a generalist parasitoid with a wide host utilization of many fly species, whereas the other Nasonia species are specialists (4, 10). Using genomic tools, a major host preference locus has been mapped to a region of ~2 cM (10), suggesting that in host use between the species may be accompanied by rapid evolution of venom proteins. The large venom protein set found in Nasonia with diverse physiological effects (40) and abundance of parasitoid species (1, 2) suggests that parasitoids may contain a rich venom pharmacopeia of potential new drugs.

A suite of genetic tools and resources is available or under development for the Nasonia system (4, 11, 28), and the genome resources presented here can be used for fine-scale mapping (6, 9–11) and positional cloning (6) of QTLs. By combining haploid genetics, ease of rearing, short generation time, systematic RNAi, interfertile species, and new genome resources for three species, Nasonia shows promise as a genetic model system for evolutionary and developmental genetics. Genome resources described here and our resulting enhanced understanding of parasitoid biology will also open avenues for improving parasitoid utility in biological control of pests of agricultural and medical importance.

References and Notes

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43. Genome sequencing, assembly and annotation were funded by the National Human Genome Research Institute (NHGRI US HG003273). The whole-genome shotgun project has been deposited at the DNA Databank of Japan (DDBJ)/European Molecular Biology Laboratory (EMBL)/GenBank under accession numbers AAZX00000000 (N. vitripennis), ADAP00000000 (N. giraulti), and ADAP00000000 (N. longicornis). Additional support, acknowledgments, and accession numbers are provided in the supporting online material.
Zebrafish Behavioral Profiling Links Drugs to Biological Targets and Rest/Wake Regulation

Jason Rihel,‡‡ David A. Prober,‡‡ Anthony Arvanites,‡ Kelvin Lam,‡ Steven Zimmerman,† Sumin Jang,‡ Stephen J. Haggarty,†,‡,‡‡ David Kokel,‡ Lee L. Rubin,‡ Randall T. Peterson,§,¶,%% Alexander F. Schier,†,‡,‡‡,§,¶,%%

A major obstacle for the discovery of psychoactive drugs is the inability to predict how small molecules will alter complex behaviors. We report the development and application of a high-throughput, quantitative screen for drugs that alter the behavior of larval zebrafish. We found that the multidimensional nature of observed phenotypes enabled the hierarchical clustering of molecules according to shared behaviors. Behavioral profiling revealed conserved functions of psychotropic molecules and predicted the mechanisms of action of poorly characterized compounds. In addition, behavioral profiling implicated novel factors such as ether-a-go-go-related gene (ERG) potassium channels and immunomodulators in the control of rest and locomotor activity. These findings are particularly acute for psychotropic drugs because brain activity cannot be modeled in vitro (1–3). Motivated by recent small-molecule screens that probed zebrafish developmental processes (4–7), we developed a whole organism, high-throughput screen for small molecules that alter larval zebrafish locomotor behavior. We used an automated rest/wake behavioral assay (3, 8) to monitor the activity of larvae exposed to small molecules at 10 to 30 μM for 3 days (Fig. 1A) (3). Multiple behavioral parameters were measured, including the number and duration of rest bouts, rest latency, and waking activity (i.e., activity not including time spent at rest) (Fig. 1B) (3). We screened 5648 compounds representing 3968 unique structures and 1680 duplicates and recorded more than 60,000 behavioral profiles. Of these, 547 compounds representing 463 unique structures significantly altered behavior relative to controls, according to a stringent statistical cutoff (3).

Because the alterations in behavior were multidimensional and quantitative, we assigned a behavioral fingerprint to each compound and applied clustering algorithms to organize molecules according to their fingerprints (Fig. 2A and figs. S1 to S3). This analysis organized the data set broadly into arousing and sedating compounds and identified multiple clusters corresponding to specific phenotypes (Fig. 2B to F; Fig. 3, A to C; Fig. 4, B and C; and figs. S1 to S4). Clustering allowed us to address three questions: (i) Do structural, functional, and behavioral profiles overlap? (ii) Does the data set predict links between known and unknown small molecules and their mechanisms of action? (iii) Does the data set identify unexpected new factors as regulators of rest and locomotor behavior.

Most current drug discovery efforts focus on simple in vitro screening assays. Although such screens can be successful, they cannot recreate the complex network interactions of whole organisms. These limitations are particularly acute for psychotropic drugs because brain activity cannot be modeled in vitro (1–3). Motivated by recent small-molecule screens that probed zebrafish developmental processes (4–7), we developed a whole organism, high-throughput screen for small molecules that alter larval zebrafish locomotor behavior. We used an automated rest/wake behavioral assay (3, 8) to monitor the activity of larvae exposed to small molecules at 10 to 30 μM for 3 days (Fig. 1A) (3). Multiple behavioral parameters were measured, including the number and duration of rest bouts, rest latency, and waking activity (i.e., activity not including time spent at rest) (Fig. 1B) (3). We screened 5648 compounds representing 3968 unique structures and 1680 duplicates and recorded more than 60,000 behavioral profiles. Of these, 547 compounds representing 463 unique structures significantly altered behavior relative to controls, according to a stringent statistical cutoff (3).

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ERRATUM

Post date 26 March 2010

Reports: “Functional and evolutionary insights from the genomes of three parasitoid Nasonia species” by J. H. Werren et al. (15 January, p. 343). The author list on page 343 should read as follows: John H. Werren, Stephen Richards, Christopher A. Desjardins, Oliver Niehuis, Jürgen Gadau, John K. Colbourne, and The Nasonia Genome Working Group. The list has been corrected on the online HTML page.