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but enhanced by GTP- γ S and AlF_4^- (Fig. 2, C and D), indicating that the cytoplasmic domains of β_3 and β_1 can directly interact with $\text{G}\alpha_{13}$ and that GTP enhances the interaction. The $\text{G}\alpha_{13}$ - β_3 interaction was enhanced in platelets adherent to fibrinogen, and by thrombin, which stimulates GTP binding to $\text{G}\alpha_{13}$ via GPCR (Fig. 2E). Hence, the interaction is regulated by both integrin occupancy and GPCR signaling.

To map the β_3 binding site in $\text{G}\alpha_{13}$, we incubated cell lysates containing Flag-tagged wild type or truncation mutants of $\text{G}\alpha_{13}$ (fig. S5) with GST- β_3 CD beads. GST- β_3 CD associated with wild-type $\text{G}\alpha_{13}$ and the $\text{G}\alpha_{13}$ 1 to 212 fragment containing α -helical region and switch region I (SRI), but not with the $\text{G}\alpha_{13}$ fragment containing residues 1 to 196 lacking SRI (Fig. 2F). Thus, SRI appears to be critical for β_3 binding. To further determine the importance of SRI, $\text{G}\alpha_{13}$ - β_3 binding was assessed in the presence of a myristoylated synthetic peptide, Myr-LLARRPTKGIHEY (mSRI), corresponding to the SRI sequence of $\text{G}\alpha_{13}$ (197 to 209) (21, 22). The mSRI peptide, but not a myristoylated scrambled peptide, inhibited $\text{G}\alpha_{13}$ binding to β_3 (Fig. 2G), indicating that mSRI is an effective inhibitor of β_3 - $\text{G}\alpha_{13}$ 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 $\text{G}\alpha_{13}$ SRI because $\text{G}\alpha_{13}$ binding to RhoGEFs stimulates RhoA activation, which should be inhibited rather than promoted by mSRI (22). Thus, these data suggest that β_3 - $\text{G}\alpha_{13}$ 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 the ADP ribosylation of RhoA) or Y27632, confirming the importance of $\text{G}\alpha_{13}$ -dependent inhibition of RhoA in platelet spreading. Thrombin promotes platelet spreading, which requires $\text{cdc}42/\text{Rac}$ pathways (23). However, thrombin-promoted platelet spreading was also abolished by mSRI (Fig. 3B), indicating the importance of $\text{G}\alpha_{13}$ - β_3 interaction. Thus, $\text{G}\alpha_{13}$ -integrin interaction appears to be a mechanism that mediates integrin signaling to c-Src and RhoA, thus regulating cell spreading.

To further determine whether $\text{G}\alpha_{13}$ mediates inhibition of integrin-induced RhoA-dependent contractile signaling, we investigated the effects of mSRI and depletion of $\text{G}\alpha_{13}$ on platelet-dependent clot retraction (shrinking and consolidation of a blood clot requires integrin-dependent retraction of platelets from within) (7, 8). Clot retraction was accelerated by mSRI and depletion of $\text{G}\alpha_{13}$ (Fig. 4, A and B, and fig. S6), indicating that $\text{G}\alpha_{13}$ negatively regulates RhoA-dependent platelet retraction and coordinates cell spreading and retraction. The coordinated cell spreading-retraction process is also important in wound healing, cell migration, and proliferation (24).

The function of $\text{G}\alpha_{13}$ in mediating the integrin-dependent inhibition of RhoA contrasts with the traditional role of $\text{G}\alpha_{13}$, 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, β_3 binding to $\text{G}\alpha_{13}$ was diminished at 1 min when $\text{G}\alpha_{13}$ -dependent activation of RhoA occurs, but increased after the occurrence of integrin-dependent platelet aggregation (Fig. 4, E and F). Thrombin-stimulated binding of $\text{G}\alpha_{13}$ to $\alpha_{\text{IIb}}\beta_3$ and simultaneous RhoA inhibition both require ligand occupancy of $\alpha_{\text{IIb}}\beta_3$ 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 $\alpha_{\text{IIb}}\beta_3$ as a noncanonical $\text{G}\alpha_{13}$ -coupled receptor but also a new concept of $\text{G}\alpha_{13}$ -dependent dynamic regulation of RhoA, in which $\text{G}\alpha_{13}$ 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

1. R. O. Hynes, *Cell* **110**, 673 (2002).
2. M. H. Ginsberg, A. Partridge, S. J. Shattil, *Curr. Opin. Cell Biol.* **17**, 509 (2005).
3. Y. Q. Ma, J. Qin, E. F. Plow, *J. Thromb. Haemost.* **5**, 1345 (2007).
4. S. J. Shattil, *Trends Cell Biol.* **15**, 399 (2005).
5. A. Obergfell et al., *J. Cell Biol.* **157**, 265 (2002).
6. E. G. Arias-Salgado et al., *Proc. Natl. Acad. Sci. U.S.A.* **100**, 13298 (2003).

7. P. Flevaris et al., *J. Cell Biol.* **179**, 553 (2007).
8. P. Flevaris et al., *Blood* **113**, 893 (2009).
9. N. A. Riobo, D. R. Manning, *Trends Pharmacol. Sci.* **26**, 146 (2005).
10. L. F. Brass, D. R. Manning, S. J. Shattil, *Prog. Hemost. Thromb.* **10**, 127 (1991).
11. A. Moers et al., *Nat. Med.* **9**, 1418 (2003).
12. T. Kozasa et al., *Science* **280**, 2109 (1998).
13. M. J. Hart et al., *Science* **280**, 2112 (1998).
14. B. Klages, U. Brandt, M. I. Simon, G. Schultz, S. Offermanns, *J. Cell Biol.* **144**, 745 (1999).
15. V. Senyuk et al., *Cancer Res.* **69**, 262 (2009).
16. B. S. Collier, *Blood* **55**, 169 (1980).
17. Z. Li, G. Zhang, R. Feil, J. Han, X. Du, *Blood* **107**, 965 (2006).
18. M. Gu, X. Xi, G. D. Englund, M. C. Berndt, X. Du, *J. Cell Biol.* **147**, 1085 (1999).
19. W. T. Arthur, L. A. Petch, K. Burridge, *Curr. Biol.* **10**, 719 (2000).
20. S. Tanabe, B. Kreutz, N. Suzuki, T. Kozasa, *Methods Enzymol.* **390**, 285 (2004).
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.
22. J. S. Huang, L. Dong, T. Kozasa, G. C. Le Breton, *J. Biol. Chem.* **282**, 10210 (2007).
23. C. Vidal, B. Geny, J. Melle, M. Jandrot-Perrus, M. Fontenay-Roupie, *Blood* **100**, 4462 (2002).
24. K. Moissoglu, M. A. Schwartz, *Biol. Cell* **98**, 547 (2006).
25. X. P. Du et al., *Cell* **65**, 409 (1991).
26. X. D. Ren, M. A. Schwartz, *Methods Enzymol.* **325**, 264 (2000).
27. This work was supported by grants HL080264, 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.

Supporting Online Material

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Materials and Methods
Figs. S1 to S6
References

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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 offspring consume and eventually kill it. Parasitoids

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 genetically 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 (N_{50}), 40.6% guanine plus cytosine content (GC)]. Contigs were placed with mate-pair information into 6,181 scaffolds (total size 295 Mb, N_{50} = 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^{-4} . 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, antibioticly 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 2.4% 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).

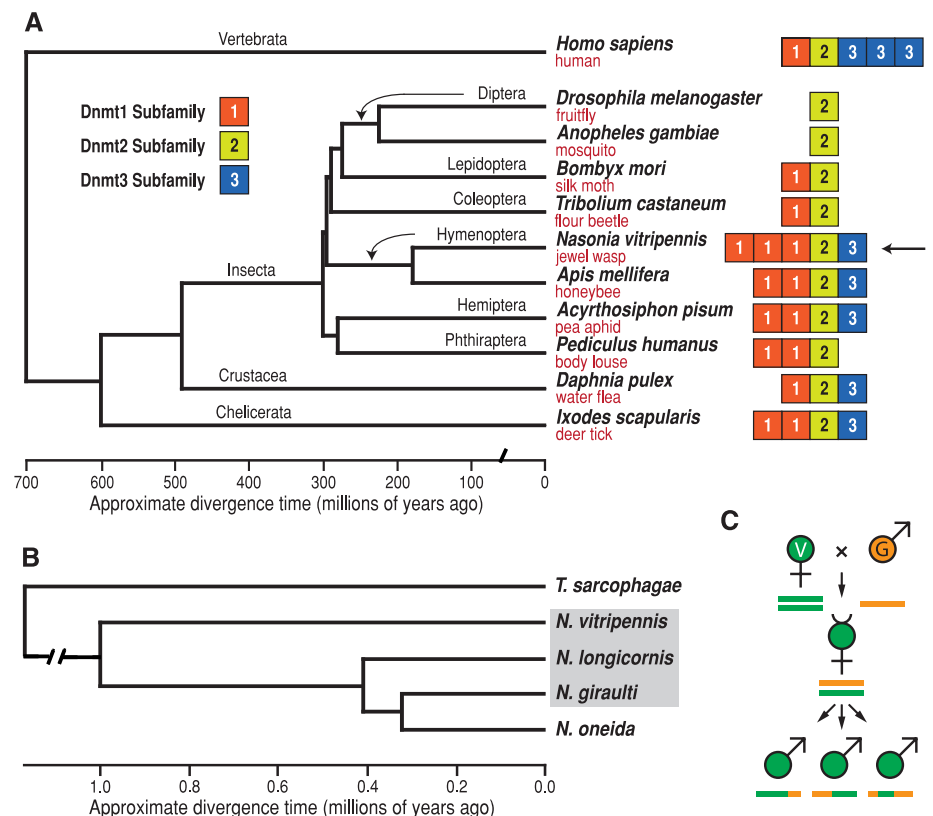


Fig. 1. Phylogenetic relationships of *Nasonia* and the DNA methylation tool kit. (A) *Nasonia* relationships to other sequenced genomes (6). Right: DNA methyltransferase subfamilies (Dnmt1, Dnmt2, Dnmt3) in these taxa. (B) Relationships among the three sequenced *Nasonia* genomes. (C) Crossing scheme used for mapping scaffolds on the *Nasonia* chromosomes and for studies of nuclear-cytoplasmic incompatibility.

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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

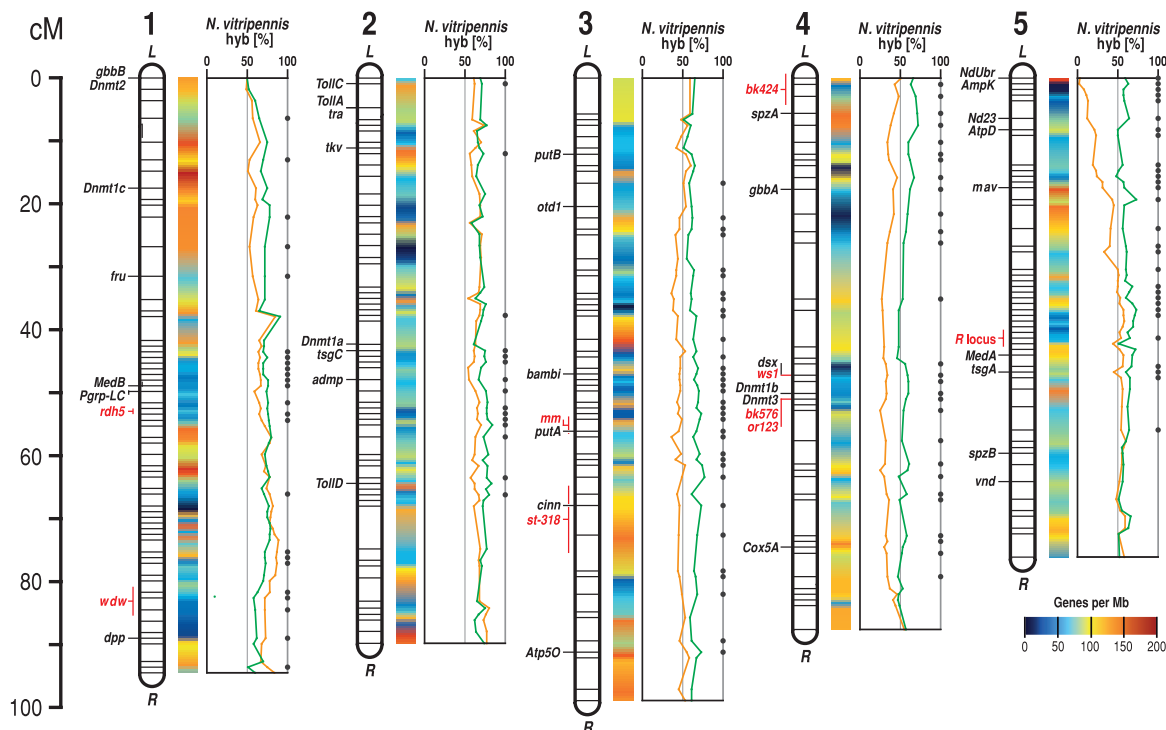
Hymenoptera-specific miRNAs (table S5). Small-RNA library sequencing confirmed 39 predicted and identified 59 additional miRNAs (table S6).

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 *Drosophila* include the transforming growth factor- β (TGF β) ligands *ADMP* and *myostatin*, and the bone morphogenesis protein (BMP) inhibitors *BAMBI* and *DAN*, but their functions in *Nasonia* are not yet known. *A. mellifera* shows an expansion of the *yellow/major royal jelly* (*yellow/MRJP*) genes that are linked to caste formation and sociality (29). *Nasonia* has the largest number of *yellow/MRJP* 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 refractoriness (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), neuropeptides (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* mitochondria (33) and studies implicating nuclear-mitochondrial incompatibilities in F2 hybrid breakdown (7, 31). For example, reciprocal crosses between *N. giraulti* \times *N. vitripennis* have identical F1 nuclear genotypes, but their mitochondrial haplotypes differ. Yet, microarray hybridization (Fig. 2) (6) 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 (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* \times *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 ($P < 0.01$).



mosome 5 and *N. giraulti* mitochondria suffer nearly 100% mortality (Fig. 2). This region contains three genes encoding mitochondrial interacting proteins, *atpD*, *ampK*, and *nadh-ubiquinone oxidoreductase* (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 organisms, 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 described in Pox viruses, where it is associated with ANK repeats and inhibits the nuclear factor κ B (NF- κ B) pathway in mammalian hosts (38). A

computational screen revealed ANK-PRANC-bearing genes in some *Wolbachia* and a related Rickettsiales (Fig. 4). Screening additional *Wolbachia* 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 sequences 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 evolutionary 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 physiological effects on hosts, including developmental 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*

Fig. 3. Distribution of recognizable *Nasonia* orthologs and *Nasonia*-specific genes among gene models with expression sequencing support (6).

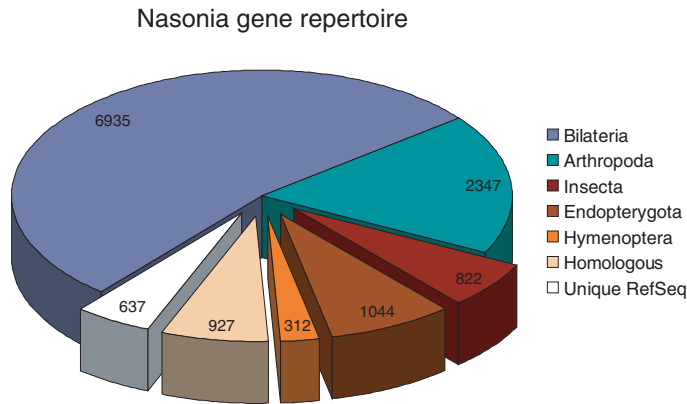
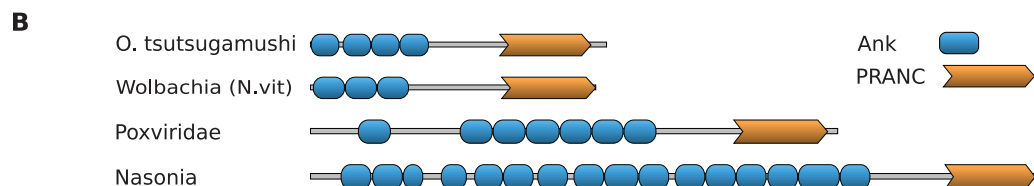
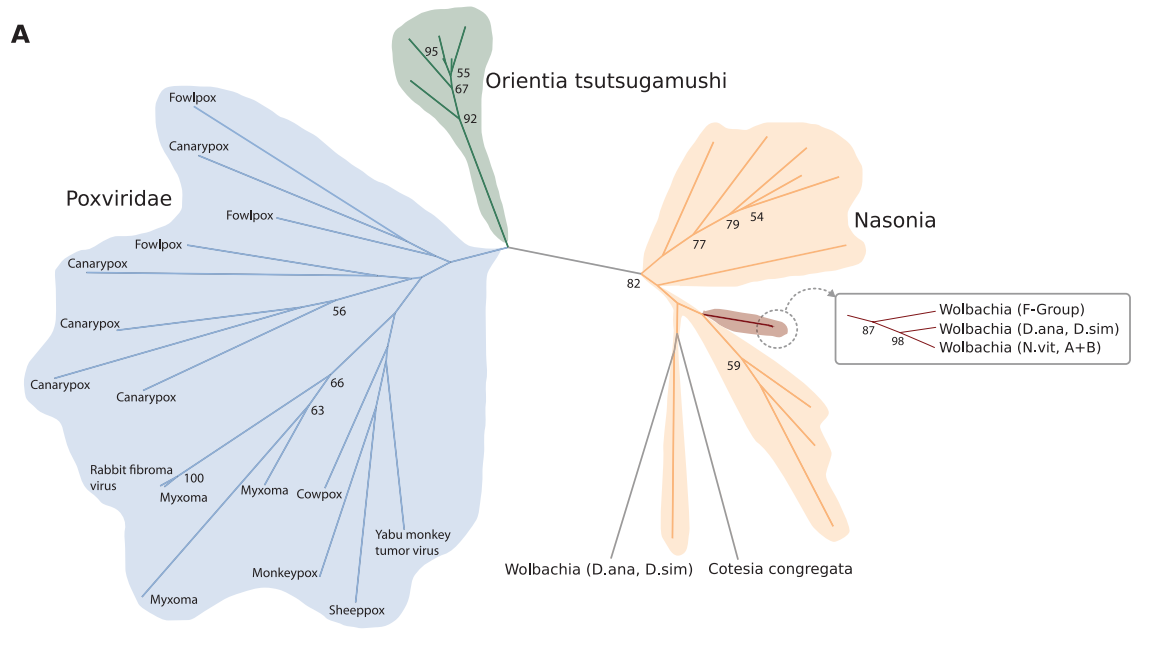


Fig. 4. PRANC domain proteins in *Nasonia*, Pox viruses, and *Wolbachia*. **(A)** Maximum-likelihood tree of PRANC-domain sequences found in Pox viruses, rickettsia (*Wolbachia* and *Orientia*), and parasitoids (*N. vitripennis* and *Cotesia congregata*). The tree was estimated using RaxML with 1000 bootstrap replicates and model settings estimated by ProtTest [see (6)]; alignment deposited in Treebase with ID SN4709]. Bootstrap values above 50% are shown by the corresponding nodes. The phylogenetic relationships suggest lateral transfer from *Wolbachia* to the *Nasonia* lineage. **(B)** Representative domain arrangements for ANK-PRANC proteins.



genes with venom features and proteomic analyses of venom reservoir tissues have uncovered a rich assemblage of 79 candidate venom proteins (table S16) (41). 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, $P < 2 \times 10^{-6}$), 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). Other genes in the *Nasonia* genome that are potentially involved in host finding include odorant binding proteins (table S17) and chemoreceptors (42), which show expansions, contractions, and pseudogenization, indicative of rapid turnover.

A suite of genetic tools and resources is available or under development for the *Nasonia* system (4, 6, 11, 28), and the genome resources presented here can be used for fine-scale mapping (6, 9–11) and positional cloning (8) of QTLs. By combining haploid genetics, ease of rearing, short generation time, systemic 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

- D. L. J. Quicke, *Parasitic Wasps* (Chapman & Hall, London, 1997).
- J. Heraty, in *Insect Biodiversity: Science and Society*, R. Tootti and P. Alder, Eds. (Wiley-Blackwell, Hoboken, NJ, 2009), pp. 445–462.
- R. Raychoudhury et al., *Heredity* 10.1038/hdy.2009.147 (2010).
- J. H. Werren, D. Loehlin, *Cold Spring Harb. Protocols* 10.1101/pdb.emo134 (2009).
- M. A. Pultz et al., *Genetics* 154, 1213 (2000).
- Materials and methods and supplementary text are available as supporting material on Science Online.
- J. A. J. Breeuwer, J. H. Werren, *Evolution* 49, 705 (1995).
- D. W. Loehlin et al., *PLoS Genet.* 10.1371/journal.pgen.1000821 (2010).
- D. W. Loehlin, L. S. Enders, J. H. Werren, *Heredity* 10.1038/hdy.2009.146 (2010).
- C. A. Desjardins, F. Perfecti, J. D. Bartos, L. S. Enders, J. H. Werren, *Heredity* 10.1038/hdy.2009.145 (2010).
- B. J. Velthuis, W. Yang, T. van Opijnen, J. H. Werren, *Anim. Behav.* 69, 1107 (2005).
- O. Niehuis et al., *PLoS ONE* 10.1371/journal.pone.0008597 (2010).
- L. Wilfert, J. Gadau, P. Schmid-Hempel, *Heredity* 98, 189 (2007).
- E. M. Zdobnov, P. Bork, *Trends Genet.* 23, 16 (2007).
- The official gene set OGS v1.2 is available at http://nasoniabase.org/nasonia_genome_consorrtium/datasets.html.
- Honeybee Genome Sequencing Consortium, *Nature* 443, 931 (2006).
- C. D. Smith et al., *Gene* 389, 1 (2007).
- L. Viljakainen, D. C. S. G. Oliveira, J. H. Werren, S. K. Behura, *Insect Mol. Biol.* 19, 27 (2010).
- B. A. Pannabakker, O. Niehuis, A. Hedley, J. Gadau, D. M. Shuker, *Insect Mol. Biol.* 19, 91 (2010).
- T. P. Jurkowski et al., *RNA* 14, 1663 (2008).
- R. Kucharski, J. Maleszka, S. Foret, R. Maleszka, *Science* 319, 1827 (2008).
- L. W. Beukeboom, A. Kamping, L. van de Zande, *Semin. Cell Dev. Biol.* 18, 371 (2007).
- N. Elango, B. G. Hunt, M. A. D. Goodisman, S. V. Yi, *Proc. Natl. Acad. Sci. U.S.A.* 106, 11206 (2009).
- L. K. Mosavi, T. J. Cammett, D. C. Desrosiers, Z. Y. Peng, *Protein Sci.* 13, 1435 (2004).
- J. A. Lynch, C. Desplan, *Nat. Protoc.* 1, 486 (2006).
- D. Gerlach, E. V. Kriventseva, N. Rahman, C. E. Vejnar, E. M. Zdobnov, *Nucleic Acids Res.* 37, D111 (2009).
- J. A. Lynch, A. E. Brent, D. S. Leaf, M. A. Pultz, C. Desplan, *Nature* 439, 728 (2006).
- A. E. Brent, G. Yucel, S. Small, C. Desplan, *Science* 315, 1841 (2007).
- M. D. Drapeau, S. Albert, R. Kucharski, C. Prusko, R. Maleszka, *Genome Res.* 16, 1385 (2006).
- N. Yapici, Y. J. Kim, C. Ribeiro, B. J. Dickson, *Nature* 451, 33 (2008).
- O. Niehuis, A. K. Judson, J. Gadau, *Genetics* 178, 413 (2008).
- J. M. Flowers et al., *Mol. Biol. Evol.* 24, 1347 (2007).
- J. van den Assem, J. Visser, *Biol. Comp.* 1, 37 (1976).
- D. C. S. G. Oliveira, R. Raychoudhury, D. V. Lavrov, J. H. Werren, *Mol. Biol. Evol.* 25, 2167 (2008).
- D. M. Rand, R. A. Haney, A. J. Fry, *Trends Ecol. Evol.* 19, 645 (2004).
- C. F. Aquadro, V. B. Dumont, F. A. Reed, *Curr. Opin. Genet. Dev.* 11, 627 (2001).
- J. C. Dunning-Hotopp et al., *Science* 317, 1753 (2007).
- S. J. Chang et al., *J. Virol.* 83, 4140 (2009).
- M. Kanehisa et al., *Nucleic Acids Res.* 36, D480 (2008).
- D. B. Rivers, Y. A. Yoder, L. Ruggiero, *Trends Entomol.* 2, 1 (1999).
- D. C. de Graaf et al., *Insect Mol. Biol.* 19, 11 (2010).
- H. M. Robertson, J. Gadau, K. W. Wanner, *Insect Mol. Biol.* 19, 121 (2010).
- Genome sequencing, assembly and annotation were funded by the National Human Genome Research Institute (NHGRI U54 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*), ADA000000000 (*N. giraulti*), and ADAP00000000 (*N. longicornis*). Additional support, acknowledgments, and accession numbers are provided in the supporting online material.
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Supporting Online Material

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Materials and Methods

SOM Text

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References

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Zebrafish Behavioral Profiling Links Drugs to Biological Targets and Rest/Wake Regulation

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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 new factors such as ether-a-go-go-related gene (ERG) potassium channels and immunomodulators in the control of rest and locomotor activity. These results demonstrate the power of high-throughput behavioral profiling in zebrafish to discover and characterize psychotropic drugs and to dissect the pharmacology of complex behaviors.

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 μ M 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. 2, B 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

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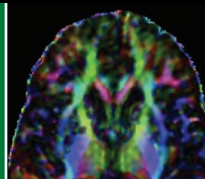
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ERRATUM

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.



LETTERS

edited by Jennifer Sills

Compelling Science Saves a River Valley



Flathead River

THE FLATHEAD RIVER, WHICH originates in British Columbia and flows into Montana, is considered one of America's wildest rivers. Its water quality is pristine, it harbors abundant and diverse aquatic life, and it sustains the full complement of mid- to large carnivores that have lived there since the 1800s. Time and time again since the mid-1970s, the river has been threatened by British Columbia's plans to strip mine for coal. In 2007, British Petroleum announced plans for coal-bed methane development in the basin (1). The swift response, which successfully prevented

these plans from coming to fruition, included three elements: a careful scientific analysis, a fact-finding mission that respected the scientific input, and a productive diplomatic relationship that resulted in policy changes.

First, the United States responded by developing a science team to conduct a comprehensive environmental assessment of water quality, aquatic food webs, habitat, native fish, and wildlife. The science was compelling. A comparison between data collected from the Flathead and from the neighboring Elk River, the site of more than 50 years of open-pit coal mining, showed that waters of the Elk basin were significantly more polluted than those of the Flathead: Elk basin had more than 1000 times the nitrates, 100 times the sulfates, and 10 times the selenium concentrations. Similarly, aquatic food webs in coal mine-affected waters lost biodiversity as many pollution-sensitive species disappeared (2). In contrast, the pristine water and aquatic habitats in the Flathead support migratory populations of endangered species such as bull trout and nonhybridized westslope cutthroat trout (3).

In September 2009, a joint United Nations Educational, Scientific, and Cultural Organization (UNESCO)/International Union for Conservation of Nature fact-finding mission visited the Flathead in Montana and British Columbia to investigate whether the proposed mining was a threat to Waterton-Glacier International Peace Park, a UNESCO-designated World Heritage Site and Biosphere Reserve. The UNESCO mission listened carefully to the scientists' results. Their report concluded that mining in the Flathead would be "incompatible" with Waterton-Glacier as a World Heritage Site (4).

Finally, diplomacy at the state/provincial level and at the federal level between the United States and Canada was developed through personal relationships and mutual interest. Policymakers in both countries respected the scientific and fact-finding analyses. As a result, on 18 February 2010, Premier Gordon Campbell of British Columbia and Governor Brian Schweitzer of Montana signed an accord to prohibit coal mining, coal-bed methane extraction,

and gas and oil exploration and development in the transboundary North Fork of the Flathead River Basin.

Throughout this process, scientific results played a central role in providing the backbone for resolute policy and the case for relentless political pressure. This healthy precedent will allow science to continue to inform policy as Canadian and U.S. officials work together to develop a natural-resource policy that protects this remarkable, shared ecosystem. We believe that this case will stand as an international model in which the natural goods and services provided by a World Heritage Site and Biosphere Reserve were ultimately valued over the commodities of natural-resource extraction.

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References

1. J. Mann, "Coal-bed permits sought," *The Daily Inter Lake*, 9 August 2007; www.dailyinterlake.com/news/local_montana/article_a58f4503-95e5-5b93-a73e-06319bc91e47.html.
2. F. R. Hauer, E. K. Sexton, "Transboundary flathead water quality and aquatic life: Biennial report" (Flathead Basin Commission, Kalispell, MT, 2010).
3. C. C. Muhlfeld, T. E. McMahon, D. Belcer, J. Kershner, *Can. J. Fish Aquat. Sci.* **66**, 1153 (2009).
4. P. R. Dingwall, K. Rao, "Report of the reactive monitoring mission" (UNESCO World Heritage Centre, International Union for Conservation of Nature, Waterton-Glacier International Peace Park, 2009).

Asian Test-Score Culture Thwarts Creativity

ASIA HAS BEEN HAILED AS THE NEXT GLOBAL science player as fast-growing Asian economies invest heavily in science and technology to drive further growth. However, Asian science will continue lagging behind the West because the Asian education system does not nurture

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Shedding light
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the creativity and thinking skills required in successful scientists.

In Asia, high scores determine the future career of the students as well as which schools get more funding. Teachers, under pressure to maintain their school's scoring record, teach to the test and organize extra classes for exam drills. Parents ferry children as young as first grade to centers for private tuition and exam preparation after school and on weekends. In Singa-

pore, a 2008 poll found 97 out of 100 students enrolled in private tuition (1). Last year, the test preparation industry was worth \$16.3 billion—36% of the public education expenditure—in South Korea (2), a country where, on college entrance exam day, parents pack churches and temples to pray and flights are rescheduled to reduce noise (3). Amidst pressure from long school days and

heavy homework, the Asian student's most intellectually demanding work is memorizing facts for regurgitation. The product of this educational culture is deficient in the inquiry, investigation, and reasoning skills needed for scientific discovery.

My experience with aspiring local graduate students has been that while motivation and work rate is usually high, they are weak at seeing connections in the published literature, extrapolating ideas, and generating hypotheses. The fact that they are top test-takers suggests that the Asian education system does not foster scientific talent.

East Asians have been described as strong in absorbing existing knowledge and adapting existing technology, but weak in making original contributions to basic science (4). A radical transformation of the educational culture must happen before homegrown Asian science can challenge Western technological dominance.

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LIFE IN SCIENCE

Anaconda!

For years a 25-foot-long reticulated python, known as Samantha, highlighted my herpetology class's visits to the Bronx Zoo. What would it be like, I'd wonder, to live in a world of mainly chemical, tactile, and infrared sensations, with one's head so far from one's tail? How would her presence affect other animals?

Despite decades of studying snakes, I lacked field experience with the few giant species until one evening in 2007, when—with my wife and her Brazilian research collaborators—I entered a Mato Grosso swamp to collect treefrogs. Our headlamps sliced back and forth through the knee-deep wetland, its vegetation glistening under a poignantly full moon.

Suddenly, I noticed staggered, dark coffee saucers winding past my left boot, like black leaves twisting in an olive current, and after a few dissonant seconds my brain flashed in recognition: a green anaconda! As her tail came into view, I grabbed with one hand, whereupon she jerked loose and reappeared several feet away. I took a few steps and lightly grasped the massive torso, worried that restraint would provoke constriction. As the enormous creature slid through my hands, I seized the 3-inch-thick tail and pulled up hard.

The next few seconds were chaotic. Her body glinted like a submarine parting the moonlit waters, then arched sideways, and I wondered if big toothy jaws were swinging our way. Instead the snake—which must have been 15 to 20 feet long—strained forward, sliding against my left hand, and I struggled backward clutching the tail in my right. Her skin was slick and tough, neither cold nor slimy. She moved with muscular confidence, as if I were a momentary distraction.

Inexorably the huge snake pulled free. But thanks to that night, I more viscerally appreciate how she could appear as benign as leaves and stones on a pond bottom. I now know why a deer



or a monkey lingering at the water's edge might not detect an anaconda's stealthy presence. There are no sounds as she approaches, no scented warnings from upwind, and sinuous ripples in the swamp grass could just as easily be a storm's breezy prelude. I now understand why complacency doesn't bode well in the home of giant serpents.

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References

1. M. Toh, "Tuition nation," *The Straits Times*, 15 June 2008; www.straitstimes.com/print/Free/Story/STIStory_248124.html.
2. C. Sang-Hun, "Tech company helps South Korean students ace entrance tests," *The New York Times*, 2 June 2009, p. B4; www.nytimes.com/2009/06/02/business/global/02cram.html.
3. S.-S. Park, "Exam sees Korea engaged in collective prayer," *The Korea Times*, 12 November 2009; www.koreatimes.co.kr/www/news/nation/2009/12/117_55389.html.
4. S. Kanazawa, *J. Evol. Psychol.* **4**, 120 (2006).

CORRECTIONS AND CLARIFICATIONS

News Focus: "The tangled roots of agriculture" by M. Balter (22 January, p. 404). The first paragraph refers to "hunter-gatherers in what is now Israel, Jordan, Syria, and Lebanon." This list, although not comprehensive, also could have included the Palestinian territories, as a few sites are within those borders.

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.

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

EDITOR'S NOTE

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