

Systematic discovery of nonobvious human disease models through orthologous phenotypes

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Biologists have long used model organisms to study human diseases, particularly when the model bears a close resemblance to the disease. We present a method that quantitatively and systematically identifies nonobvious equivalences between mutant phenotypes in different species, based on overlapping sets of orthologous genes from human, mouse, yeast, worm, and plant (212,542 gene-phenotype associations). These orthologous phenotypes, or phenologs, predict unique genes associated with diseases. Our method suggests a yeast model for angiogenesis defects, a worm model for breast cancer, mouse models of autism, and a plant model for the neural crest defects associated with Waardenburg syndrome, among others. Using these models, we show that *SOX13* regulates angiogenesis, and that *SEC23IP* is a likely Waardenburg gene. Phenologs reveal functionally coherent, evolutionarily conserved gene networks—many predating the plant-animal divergence—capable of identifying candidate disease genes.

angiogenesis | bioinformatics | evolution | gene-phenotype associations | homology

Biochemical and molecular functions of a given protein are generally conserved between organisms; this observation is fundamental to biological research. For example, in x-ray crystallography studies, one can often choose the organism from which the protein is most easily crystallized to facilitate the study of the protein's biochemical function. On the other hand, even with a conserved gene, disruption of function may give rise to radically different phenotypic outcomes in different species. For example, mutating the human *RB1* gene leads to retinoblastoma, a cancer of the retina, yet disrupting the nematode ortholog contributes to ectopic vulvae (1, 2). Thus, although a gene's "molecular" functions are conserved, the "organism-level" functions need not be. When a conserved gene is mutated, the resulting organism-level phenotype is an emergent property of the system. This bedrock principle underlying the use of model organisms not only allows us to study important aspects of human biology using mice or frogs, but also permits exploration of inherently multicellular processes, such as cancer, using unicellular organisms like yeast.

Within this paradigm, once a molecular function has been discovered in one organism, it should be predictable in other organisms: *GSK3* homologs in yeast are kinases, and such *GSK3* homologs in every other organism will generally be kinases. In contrast, the emergent organism-level phenotypes are far less predictable between organisms, in part because relationships between genes and phenotypes are many-to-many. Manipulation of *GSK3* perturbs nutrient and stress signaling in yeast, anteroposterior patterning and segmentation in insects, dorsoventral patterning in frogs, and craniofacial morphogenesis in mice (3–5). Recognizing functionally equivalent organism-level phenotypes between model organisms can therefore be nonobvious, especially across large evolutionary distances.

However, the ability to recognize equivalent phenotypes between different model organisms is important for the study of human diseases. Given the success of studies in model systems (genes and phenotypes have been associated in model organisms at a far higher rate than for humans) (Fig. 1A), it seems likely that useful and

tractable models for human disease await discovery, currently hidden by differences in the emergent appearance of phenotype in diverse model organisms. Although a framework exists for discussing complex gene-phenotype relationships across evolution, we lack simple—and importantly, quantifiable—methods for discovering new gene-phenotype relationships from existing data.

As a foundation for a quantifiable approach to identifying equivalent phenotypes, we introduce the notion of orthologous phenotypes (phenologs), defined as phenotypes related by the orthology of the associated genes in two organisms. Phenologs are the phenotype-level equivalent of gene orthologs. Two phenotypes are thus said to be orthologous if they share a significantly larger set of common orthologous genes than would be expected at random (i.e., are enriched for the same orthologous genes) (Fig. 1B), even if the phenotypes may appear dissimilar.

Phenologs, therefore, are evolutionarily conserved outputs arising from disruption of any of a set of conserved genes (Fig. 1B, green and blue). These outputs manifest as different traits or defects in different organisms because of the organism-specific roles played by each set of genes. One example, noted above, is the human retinoblastoma eye cancer and the *Caenorhabditis elegans* ectopic vulvae. These phenotypes are orthologous, as failure of equivalent genes (the Rb pathway)—performing conserved molecular functions but in different contexts—leads to different phenotypes in the different organisms (1, 2).

By quantifying the equivalence of mutational phenotypes between different organisms, we demonstrate that orthologous phenotypes may be found objectively, and that these phenologs suggest nonobvious models for human disease. We demonstrate the power of this approach by defining a unique yeast model that effectively predicts vertebrate angiogenesis genes and a plant model that predicts genes involved in vertebrate craniofacial defects that are associated with human congenital malformations.

Results and Discussion

Phenologs are identified by assembling known gene-phenotype associations for two organisms—considering only genes that are orthologous between the two organisms—and searching for interorganism phenotype pairs with significantly overlapping sets of genes. Significance is derived from three observations: (i) the total number of orthologs in organism 1 that give rise to phenotype 1; (ii) the total number of orthologs in organism 2 that give rise to phenotype 2; and (iii) the number of orthologs shared between these two sets. Formally, significance of a phenolog is calculated

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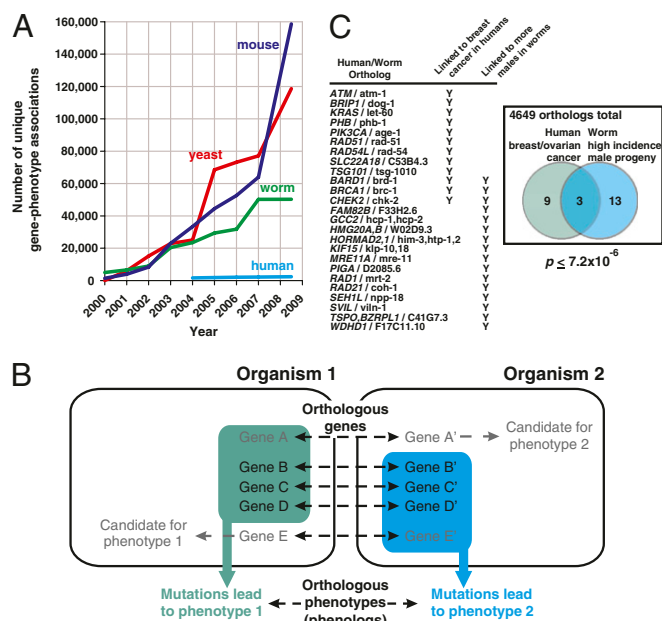


Fig. 1. Number of unique gene-phenotype associations, identification of phenologs, and the example of a worm model of breast cancer. (A) The rate of associating genes to organism-level phenotypes in model organisms greatly exceeds that in humans (data from refs. 8–11, 14). Thus, appropriate mapping of model organism phenotypes to human diseases could significantly accelerate discovery of human disease gene associations. Orthologous phenotypes (phenologs) offer one such approach. (B) Phenologs can be identified based on significantly overlapping sets of orthologous genes (gene A is orthologous to A', B to B', etc.), such that each gene in a given set (green box or cyan box) gives rise to the same phenotype in that organism. The phenotypes may differ in appearance between organisms because of differing organismal contexts. As gene-phenotype associations are often incompletely mapped, genes currently linked to only one of the orthologous phenotypes become candidate genes for the other phenotype; that is, the gene A' is a new candidate for phenotype 2. (C) An example of a phenolog mapping high incidence of male *C. elegans* progeny to human breast/ovarian cancers (details in text).

from the hypergeometric probability of observing at least that many shared orthologs by chance (*SI Materials and Methods*).

Fig. 1C shows an example of these observations: the set of human-worm genes associated with X-linked breast/ovarian cancer in human significantly overlaps the set of genes whose mutations lead to a high frequency of male progeny in *C. elegans*. Male *C. elegans* are determined by a single X chromosome, hermaphrodites by two copies; thus, X chromosome nondisjunction leads to higher frequencies of males (6). Human nondisjunction cancers may derive from X chromosome abnormalities (7), supporting the notion that this phenolog is identifying a useful disease model. Human orthologs of the 13 additional genes associated with this worm trait are thus reasonable candidate genes for involvement in breast/ovarian cancers. Nine of these genes were not yet linked to breast cancer in the databases we employed, but could be confirmed as such in the primary literature (Table S1). The remaining four genes (*GCC2*, *PIGA*, *WDHD1*, *SEH1L*) are thus implicated as breast cancer candidate genes. This rate of literature confirmation is 268-fold higher than that expected based on the current annotation rate in the Online Mendelian Inheritance in Man database (8), providing significant support for the utility of the worm phenotype to predict and suggest additional genes relevant to human breast cancer. (Note that the estimated fold-improvements we present here depend upon consistent literature curation and, thus, should be interpreted cautiously.)

Systematic Discovery of Phenologs. To systematically discover phenolog relationships, we collected from the literature a set of 1,923 human disease-gene associations (8), 74,250 mouse gene-phenotype associations (9), 27,065 *C. elegans* gene-phenotype associations (10), and 86,383 yeast gene-phenotype associations (11–14). The dataset spans ~300 human diseases and > 6,000 model organism phenotypes. With these data and the sets of orthologous gene relationships between each pair of organisms (15), we quantitatively examined the overlap of each interorganism phenotype pair, measuring their significance (Fig. 2A). To correct for testing multiple hypotheses, we repeated all analyses 1,000 times with randomly permuted gene-phenotype associations, then calculated a false-discovery rate (FDR) based upon the observed null distribution of scores (Fig. 2B and Fig. S1). We observed 154 significant phenologs (5% FDR) between human diseases and yeast mutational phenotypes, 3,755 between human and mouse, 147 between mouse and worm, 119 between mouse and yeast, 206 between yeast and worm, and 9 between human and worm (the low number stems from limited mutational data in both species) (Fig. 2C).

Many specific, intuitively obvious, phenologs were revealed by this analysis, especially for the comparison of mouse and human phenotypes. Our analysis recapitulates many known mouse models of disease, providing an important positive control for our approach; Table 1 lists other specific examples of both known and previously undescribed equivalences. For example, one of the most significant phenologs identified between human disease and mouse mutational phenotypes is that linking Bardet-Biedl syndrome with four mouse traits, each of which relates to the disruption of ciliary function (abnormal brain ventricle/choroid plexus morphology,

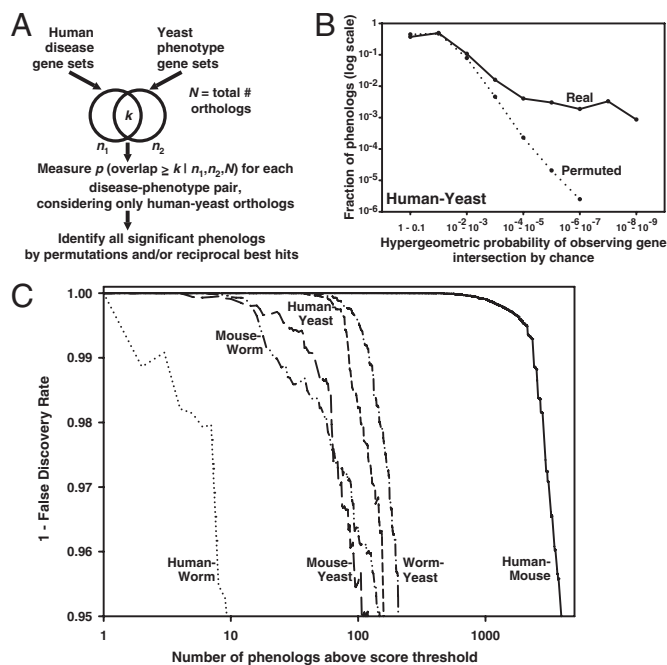


Fig. 2. Systematic identification of phenologs. (A) For a pair of organisms, sets of genes known to be associated with mutational phenotypes are assembled, considering only orthologous genes between the two organisms. Pairs of mutational phenotypes—one phenotype from each organism, each associated with a set of genes—are then compared to determine the extent of overlap of the associated gene sets, calculating the significance of overlap by the hypergeometric probability. Comparison of the distribution of observed probabilities with those derived from the same analysis following permutation of gene-phenotype associations reveals that many more orthologous phenotypes are observed than expected by random chance, as shown in B for the case of the human-yeast comparison (also Fig. S1), and summarized for each organism pair in C.

small hippocampus, enlarged third ventricle, absent sperm flagella; all $P \leq 10^{-11}$), consistent with the apparent molecular defects in Bardet-Biedl syndrome (16). These phenologs thus suggest mouse ciliary defects provide a powerful model for studying human Bardet-Biedl syndrome, consistent with its recognized utility in this regard. Similarly, human cataracts are observed to be phenologous to mouse cataracts ($P \leq 10^{-24}$), human obesity is phenologous to mouse obesity ($P \leq 10^{-14}$), human deafness to mouse deafness ($P \leq 10^{-29}$), human retinitis to mouse retinal degeneration ($P \leq 10^{-26}$), and human goiter to mouse enlarged thyroid glands ($P \leq 10^{-8}$). Thus, the calculation of phenologs correctly identifies many known mouse models of human diseases and therefore has the potential to identify new models. More generally, cross-validated tests of phenologs confirm their strong predictability for genes associated with a substantial portion of human diseases (Fig. S2).

Much of the powerful conceptual framework established for determining homology and orthology for gene sequences may also be applicable to phenologs. For example, many of the algorithmic approaches used to identify orthologous genes might also be applied to the identification of phenologs. We explored this notion for one effective and easily automated approach to identify orthologous sequences, the reciprocal best hit (RBH) strategy. The RBH criterion holds that genes X and Y are orthologs if genes X and Y are the most similar to each other (reflexively) when searched genome-wide. We adapted the RBH criterion to the identification of phenologs to identify the most equivalent phenotypes between two organisms from among those assayed, by asking if the phenotypes have the most significant (lowest P value) gene overlaps with each other when searched against all phenotypes in their respective organisms. Such analysis gives a second criterion for identifying phenologs, useful for legitimate phenologs with poor P values because of limited phenotypic data sets. Examples of such RBH phenologs are indicated in Table 1.

Mouse and Yeast Phenologs Predict Unique Angiogenesis Genes. The power of the phenolog framework lies in discovery of nonobvious disease models. We identified just such a phenolog between abnormal angiogenesis in mutant mice and reduced growth rate

of yeast deletion strains when grown in the hypercholesterolemia drug lovastatin (8 mouse, 67 yeast, 5 shared genes, $P \leq 10^{-6}$) (Fig. 3A). This observation suggests that budding yeast, which obviously lack blood vessels, could potentially model the genetics of mammalian vasculature formation and could be used to identify previously unrecognized genes affecting this process.

We identified five shared genes between these processes. In yeast, they are the MAP kinases *SLT2*, *PBS2*, and *HOG1*, the calcineurin B gene *CNBI*, and the uncharacterized gene *VPS70*. Strikingly, mutations of their mouse orthologs (*MAPK7*, *MAP2K1*, *MAPK14*, *PPP3R1*, and the prostate-specific membrane antigen *PSMA*, respectively) all confer strong angiogenesis defects (e.g., *MAPK7* deletion causes defective blood vessel and cardiac development) (17), and ablation in adult mice leads to leaky blood vessels (18). Similarly, *PSMA* regulates angiogenesis by modulating integrin signal transduction (19). Thus, this conserved set of genes was alternately repurposed to regulate cell wall stress and biogenesis in yeast cells (20) or to regulate proper formation and maintenance of blood vessels in mice.

Phenologs are most important for their ability to map gene-phenotype associations from one organism to another. In other words, phenologs suggest unobserved associations in one organism from observed associations in another (Fig. 1B, gene E and gene A'). Thus, examination of phenologs should substantially improve the rate of discovery of new genes over the rate expected by chance (here ~ 1 in 267, as estimated from the frequency of known angiogenesis genes). We might a priori expect a discovery rate comparable to the fraction of genes already verified by the phenolog; for the yeast angiogenesis model, a likely discovery rate of ~ 1 in 13, for a ~ 21 -fold higher discovery rate than random expectation. Our model therefore asserts that some of the 62 additional genes associated with lovastatin sensitivity in yeast would be predicted to be involved in angiogenesis. In fact, literature confirms three of the corresponding mouse genes to function in angiogenesis: the known target of lovastatin, HMG-CoA reductase, whose role in angiogenesis has been previously observed (21), the sirtuin *SIRT1*, whose disruption in zebrafish and mice caused defective blood vessel formation and blunted ischemia-

Table 1. Examples from the >6,200 significant phenologs detected among human diseases and mouse, yeast, worm, and *Arabidopsis* mutant phenotypes

	Phenotype ₁		Phenotype ₂	n_1	n_2	k	P value	PPV
Hs	Cataracts	Mm	Cataracts	19	47	11	6×10^{-24}	1.00
Hs	X-linked conductive deafness	Mm	Circling	47	50	12	2×10^{-20}	1.00
Hs	Bardet-Biedl syndrome	Mm	Absent sperm flagella	11	5	4	8×10^{-13}	1.00
Mm	Lymphoma	Sc	CANR mutator high	14	11	6	1×10^{-11}	1.00
Hs	Zellweger syndrome	Sc	Reduced number of peroxisomes	8	6	4	1×10^{-9}	1.00
Hs	Susceptible to autism	Mm	Abnormal social investigation	5	16	3	1×10^{-8}	1.00
Mm	Abnormal heart development	At	Defective response to red light	25	9	4	3×10^{-7}	1.00
Hs	Refsum disease	At	Defective protein import into peroxisomal matrix	4	5	2	1×10^{-5}	1.00
Mm	Absent posterior semicircular canal	At	Shade avoidance defect	2	4	2	1×10^{-6}	0.99
Mm	Spleen hypoplasia	Sc	Uge (enlarged cells)	5	16	3	3×10^{-6}	0.99
Mm	Gastrointestinal hemorrhage	Ce	Abnormal body wall muscle cell polarization	6	3	2	4×10^{-6}	0.98
Hs	Mental retardation	At	Cotyledon development defects	13	5	2	1×10^{-4}	0.98
Hs	Congenital disorder of glycosylation	Sc	CID 604586 sensitive	10	25	3	2×10^{-4}	0.98
Hs	Hemolytic anemia	Sc	Hydroxyurea sensitive	11	23	3	2×10^{-4}	0.98
Hs	Amyotrophic lateral sclerosis	Sc	Increased resistance to wortmannin	2	34	2	2×10^{-4}	0.97

n_1 indicates the number of orthologs in organism 1 with phenotype₁, n_2 the number in organism 2 with phenotype₂, and k the number in both sets. The significance of each phenolog is assessed by the hypergeometric probability (P value), the positive predictive value (PPV) when considering multiple testing ($1 - \text{FDR}$), and the reciprocal best-hit criterion (bold text). At, *Arabidopsis*; Ce, worm; Hs, human; Mm, mouse; Sc, yeast.

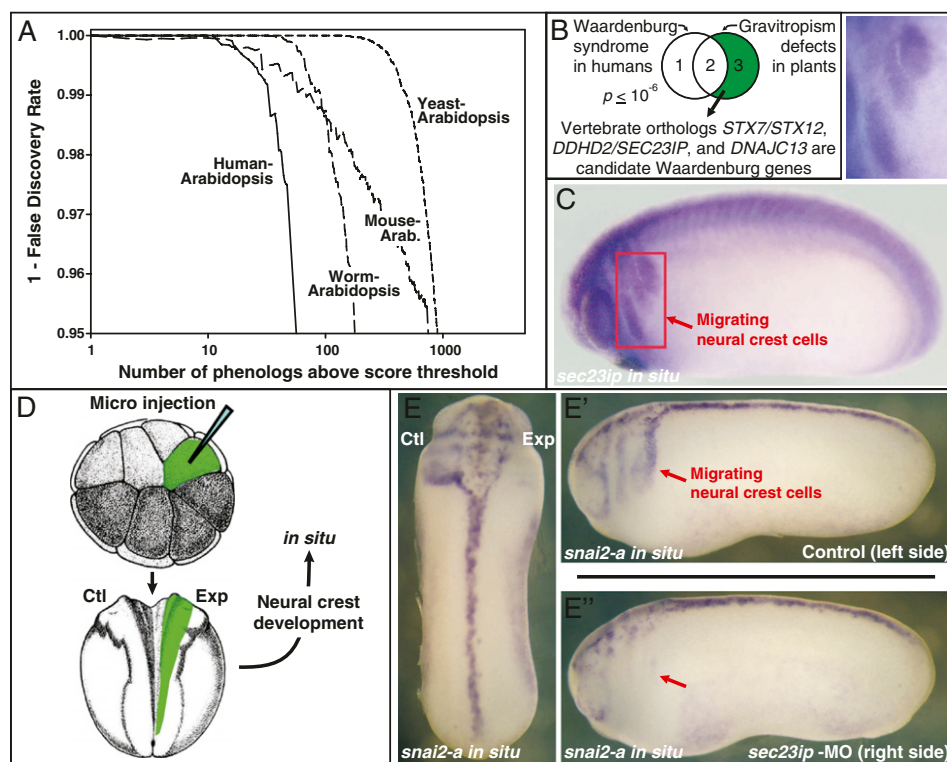


Fig. 4. Phenologs reveal plant models of human disease, including a model of Waardenburg syndrome (WS) neural crest defects. (A) Many orthologous phenotypes are observed between *Arabidopsis* and worms, yeast, mouse, and humans, with hundreds more than expected by chance. Many mammalian/plant phenologs relate to vertebrate developmental defects, including models for WS and other birth defects. (B) Considering only human/*Arabidopsis* orthologs, the three known WS genes significantly overlap the five genes associated with negative gravitropism defects in *Arabidopsis*. The plant gene set suggests unique candidate WS genes. (C) In situ hybridization versus candidate *sec23ip* in developing *Xenopus* embryos confirms neural crest cell expression. (D) Unilateral morpholino knockdown of *sec23ip* induces (E) defects in neural crest cell migration on the side with the knockdown (E'') but not the control side (E'), measured using in situ hybridization versus two independent markers of neural crest cells, *snai2-a* (defects observed in 23 of 35 animals tested) and *twist* (8 of 14 animals tested) (Fig. S7). Such defects are rare in untreated control animals and off-target morpholino (OM) knockdowns (0 of 21 control animals tested with *snai2-a*; 1 of 14 OM animals tested with *snai2-a*; 0 of 14 OM animals tested with *twist*).

and diverse human diseases, including cancers, peroxisomal disorders, such as Refsum disease and Zellweger syndrome, and a variety of birth defects (Table 1).

We observed a striking plant-human phenolog relating negative gravitropism defects to Waardenburg syndrome (Fig. 4B). This congenital syndrome stems from defects in the developing neural crest and is characterized by craniofacial dysmorphology, abnormal pigmentation, and hearing loss [in fact, it accounts for 2–5% of cases of human deafness (27)]. In particular, this phenolog suggested that a set of three vesicle trafficking genes involved in directing plant growth in response to gravitational cues might also serve to direct neural crest cell migration and differentiation in developing animal embryos.

Encouragingly, one of the identified proteins (STX12) is known to interact with the protein encoded by the *pallid* gene in mice (28), whose mutational phenotypes, including pigmentation and ear defects, are consistent with Waardenburg syndrome (29). The remaining two proteins had no support in the literature, and we therefore evaluated the three mammalian orthologs of these genes by whole mount in situ hybridization in developing *Xenopus* embryos. Strikingly, we found that *sec23ip* was prominently expressed in migrating neural crest cells (Fig. 4C). We used targeted microinjection of *sec23ip* morpholinos to knock down this gene specifically in the neural crest. Unilateral targeting of *sec23ip* MOs (Fig. 4D) resulted in marked defects in neural crest cell migration patterns specifically on the injected side (Fig. 4E and Fig. S7), thus confirming a role for this gene in neural crest cell development. Notably, SEC23IP physically associates with SEC23, a component

of the COPII complex that controls ER-to-Golgi trafficking, and mutations in *SEC23* underlie Cranio-lenticular-sutural dysplasia, another congenital human disease related to neural crest development (30). Thus, *SEC23IP*, identified here on the basis of orthology to plant gravitropism defects, is both a promising candidate gene for Waardenburg syndrome and also provides insights into the emerging link between COPII function and craniofacial development in vertebrates (31). Our success rate of one in two for finding Waardenburg-relevant genes represents a 550-fold improvement over the current annotation rate of ~1 in 1,100 genes. Notably, in spite of the extremely dissimilar associated phenotypes, phenologs can identify functionally coherent gene sets that predate the divergence of plants and animals.

Phenologs Reveal Deeply Homologous Modular Subnetworks. Phenologs imply that although phenotypes diverge, the orthology of the underlying gene networks—and the networks' immediate functional output—is conserved. We might therefore expect genes involved in a given phenolog to represent a coherent biological module, and thus to be highly interconnected in gene networks. Moreover, we might expect that the genes already confirmed to show the signature phenotypes in both organisms (e.g., the intersection labeled by *k* in Fig. 2A) would be even more highly interconnected than the genes associated with the signature phenotype in only one organism; these latter genes might or might not belong to this subnetwork, as multiple mechanisms might give rise to the phenotype. Evidence in current gene networks of more linkages among the genes in each such intersection would support this

notion of phenologs recapitulating modular subnetworks. We therefore systematically tested all significant phenologs involving yeast and worm genes for the genes' connectivity in available functional networks (32). We find the network connectivity of genes in phenolog intersections to be significantly higher ($P < 0.0001$; Wilcoxon signed-rank) than the phenolog genes outside of the intersections, which nonetheless show significantly higher network connectivity than random size-matched gene sets ($P < 0.0001$) (Fig. S8). Additional tests confirm that genes in phenolog intersections are no more enriched for homologous genes than are phenolog genes outside of the intersections (Fig. S9), ruling out trivial discovery of "deep paralogs." These observations indicate that phenologs identify evolutionarily conserved subnetworks of genes relevant to particular phenotypes or diseases, yet still predicting as yet undiscovered candidate genes significantly better than random expectation. Phenologs may inherently identify systems such as those found by aligning protein interaction networks across species (33). Indeed, direct searches for evolutionarily conserved subnetworks composed largely of genes with similar phenotypes might provide an alternate strategy for phenolog discovery.

Conclusions

Phenologs reflect the innate modularity of gene systems and identify adaptive reuse of those systems, creating a rich framework for comparing mutational phenotypes with potential for finding nonobvious models of human disease. Cross-validated tests indicate phenologs show utility for roughly one-third to one-half of tested human genetic diseases (Fig. S2). Given a phenolog for a human disease, any approach for associating more genes with the model organism trait (e.g., a genetic screen) will suggest additional new human disease gene candidates. In addition to associating unique genes with modeled diseases, such models can provide mechanistic understanding in simplified model organisms for understanding aspects of more complex human diseases.

Phenologs thus bridge the molecular definitions of homologous and orthologous genes (34) with classic definitions of homologous structures from Owen (35) and Darwin (36), deriving from considerations both of gene heredity and of the traits/structures affected by perturbing the genes, concepts falling within the general field of evolutionary developmental biology (evo-devo) (37). The conserved gene systems revealed by the plant-vertebrate phenologs illustrate a more ancient homology than the "deep homology" of metazoans that is currently a focus of evolutionary developmental biology (26). These phenologs should bring attention to the potentially extensive molecular toolkit within the last common eukaryotic ancestor, which facilitated the parallel evolution of complex multicellular organisms. This comparative approach provides a simultaneously deeper and wider view of the evolution of life and points the way to a greater synthesis of evolutionary developmental biology and modern medicine.

Materials and Methods

Detailed information regarding the collection of phenotypes, identification of nonredundant phenotype sets, calculation of orthologs, calculation of phenologs, and tests of subnetwork modularity can be found in the *SI Materials and Methods*. Animal care met the principles and guidelines of the Institute for Laboratory Animal Research "Guide for Care and Use of Laboratory Animals" and the University of Texas at Austin Institutional Animal Care and Use Committee. Details of *Xenopus laevis* embryo manipulations and tube formation assays can be found in the *SI Materials and Methods*.

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