The Naturalist in a World of Genomics

(An American Society of Naturalists Symposium Paper)*

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ABSTRACT: Functional genomics provides new opportunities to address issues of fundamental interest in evolutionary biology and suggests many new research directions that are ripe for evolutionary investigation. New types of data, and the ability to study biological processes from a whole genome perspective, are likely to have a profound impact on evolutionary biology and ecology. To illustrate, we discuss how genomewide gene expression studies can be used to reformulate questions about trade-offs and pleiotropy. We then touch on some of the new research opportunities that the application of functional genomics affords to evolutionary biologists. We end with some brief notes about how evolutionary biology and comparative approaches will probably have an impact on functional genomics.

Keywords: genomics, quantitative genetics, trade-offs.

Although *The American Naturalist* is devoted to the conceptual unification of all the biological sciences, it has long emphasized evolution and ecology. By exploring how the methods and data of functional genomics affect evolutionary and ecological concepts and questions, we hope to stimulate the application of functional genomic techniques to those fields. We stress three main themes: the probable impact of functional genomics on evolutionary concepts, the new research programs suggested by studies of genomic function, and the reciprocal contributions that evolutionary biology and comparative approaches will probably make to functional genomics.

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First, we give some definitions and a caveat. The "genome" consists of all or a large part of the nucleic acid sequences that represent the heritable information in an organism. The "transcriptome" consists of the transcribed set of messenger RNA molecules present in the cytoplasm at the time a sample is taken. Similarly, the "proteome" consists of the set of proteins that have been translated in the tissues and individuals sampled at the time of sampling. "Functional genomics" is the study of genomic function, broadly defined, and may be approached from a variety of levels, including transcriptional and proteomic studies of particular samples of interest. Depending on the precision of the sampling scheme, the samples may range from an extract of a small part of a single individual of known sex, age, size, developmental stage, and physiological state to an extract of a mixture of many individuals of both sexes, many ages, many developmental stages, and many physiological states. Permissible inferences about function are correspondingly constrained.

Genomes of >100 prokaryotes and numerous eukaryotes have now been sequenced, and in the not-too-distant future, complete sequences of the genomes of hundreds of additional organisms of interest will be available from public resources (e.g., the National Center for Biotechnology Information [http://www.ncbi.nlm.nih.gov] and the Institute for Genome Research [http://www.tigr.org]). Whole-genome microarrays are currently available for assaying transcriptomes (fig. 1), and proteome microarrays are available as well. Prices for instrumentation are falling, analysis is increasingly automated, and software packages for bioinformatic analysis are increasing steadily in power and statistical appropriateness. This technology will soon be within the reach of any well-funded basic research program.

However, our field does not have a technology-driven history, and many in it are proud of that fact. Why be led by technology now? The answer is straightforward: genomics has the potential, in combination with other methods, to redefine and deepen our understanding of many

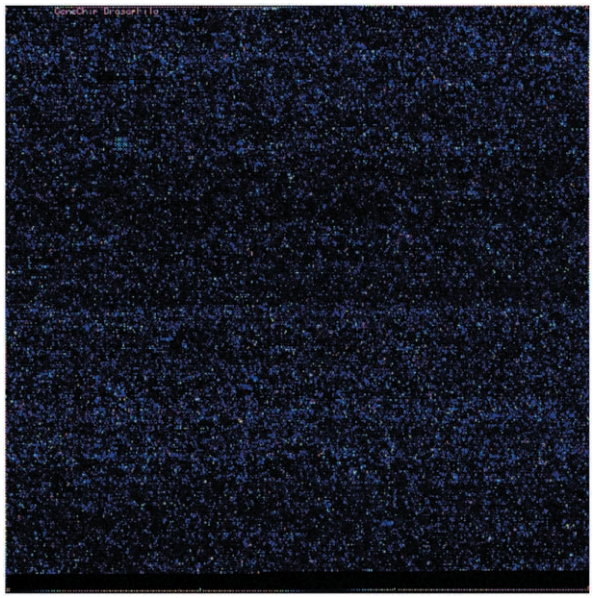

Figure 1: Expression of all known genes in the *Drosophila* genome (about 13,600) on an Affymetrix GeneChip (courtesy of S. Pletcher). Can conflicts over such whole-genome expression patterns explain trade-offs? The Affymetrix GeneChip is a microarray technology that uses photolithography to construct on a computer chip short-chain oligonucleotides that hybridize precisely to messenger RNA marked with a dye and injected onto the chip. Gene expression is measured as light intensity at the recognition site for that gene on the chip (Lockhart et al. 1996). Another microarray technology uses a robot to place primers into spotted arrays on glass slides, putting the primer for one gene into one spot and then adding the sample to be tested for all spots. A flourescent reaction yields a color and intensity of light that measures the expression of each gene (Schena et al. 1995). The chip or array is then illuminated by a laser and scanned into a computer through a confocal electron microscope.

of our central concepts. Functional genomics will be useful to naturalists, at least as it is treated in the pages of this journal, to the extent that it improves the conceptual structure of evolution and ecology and their connections to the rest of biology. We will not be led by technology; our job is to harness technology for conceptual purposes.

How we, as evolutionary biologists and ecologists, might harness this technology and, conversely, how the insights of evolutionary and comparative biology might affect the use of these technologies in other fields are the subjects of this article.

Impact on Core Concepts in Evolution and Ecology

The most important long-range impact of genomics on evolution and ecology will be changes, if any, in core concepts. At this early stage, it appears that such changes are probable in how we think about some of the fundamental parameters of classical quantitative genetics. For example, functional genomics experiments could be used to ask whether additive genetic variation primarily corresponds to variation in the protein products of structural genes. Similarly, we might investigate whether interaction variation can be related to the structure of cis-regulatory networks. To what extent will analytical and experimental techniques from functional genomics combine with or supplant methodologies developed in quantitative genetics? Will the study of quantitative trait loci (QTL) be replaced by sophisticated versions of "candidate gene" approaches (e.g., Golub et al. 1999; Huang et al. 2002) for associating gene expression profiles with phenotypes of interest? No one can answer these and many similar questions at this point, but it is at least clear that the summary parameters of quantitative genetics are now open to analysis with functional genomic techniques.

To illustrate the potential impact of functional genomic studies on key issues in evolutionary biology, we next develop an extended example in which we discuss how studies of the transcriptome might be used to cast the concepts of trade-offs and pleiotropy in new light. These concepts have played important roles in the study of research on life histories and aging (e.g., Stearns 1992), and such an illustration may help to make the claim for impacts on core concepts more convincing.

Trade-offs and Pleiotropy

It has often been inferred that trade-offs and pleiotropy are negatively correlated responses to selection, whole-organism attributes measured on populations. But we do not understand their developmental and physiological causes, and we do not know how and why they do or do not change under selection. Trade-offs and pleiotropy are black boxes located within theories that are much more explicit about mechanisms at the level of whole organisms and populations than they are about mechanisms inside organisms.

In life-history theory, if extrinsic mortality rates change in a certain manner, we predict a reallocation among growth, reproduction, and maintenance in a specific way, given trade-offs among those functions with a certain form. For example, if extrinsic adult mortality rates increase, we predict earlier maturation at a smaller size and greater allocation to reproduction early in life (Roff 1992; Stearns 1992). The trade-offs are often assumed, not measured. When they are measured, the measurements usually do not reveal what is causing them, and the theory does not predict the nature or shape of the trade-offs; they are usually imported deus ex machina from outside the theory as boundary conditions on the problem.

In the evolutionary theory of aging, genes with antag-

onistic pleiotropic effects are thought to improve performance through their impact on early life traits that make a major contribution to fitness while eroding performance through their impact on late life traits that make little contribution to fitness (Rose 1991). It has proven difficult to find such genes (Stearns and Partridge 2001), although correlated responses in traits consistent with (but not demonstrative of) antagonistic pleiotropy are common. Thus, the general idea of antagonistic pleiotropy might be correct, but we appear to have been looking for it in the wrong place or in the wrong way.

Trade-offs as Conflicts over Gene Expression

What might be the right place and the right way? Can we simplify the complex connections between genotype and phenotype to reveal readily understandable mechanisms that produce trade-offs and pleiotropy? We suggest that we define both trade-offs and antagonistic pleiotropy as conflicts between whole-organism functions over whole-genome patterns of gene expression. This can be done in straightforward steps for any species whose genome has been sequenced and for which, therefore, a whole-genome microarray can be designed and produced. The new definition places one level of intermediate structure (Stearns 1986), gene expression, between genotype and phenotype.

The first step is to ignore the intracellular details and to describe the whole-organism function of each gene as its expression response to the ecological challenges relevant to evolutionary hypotheses. Such challenges differ from species to species. To get on the list of relevant challenges, a factor must affect the reproduction and survival rates of the species in the wild. For many species such factors will include reproduction, starvation, food poisons, pathogen attack, and extreme temperatures. For some they will include strenuous exercise, humidity, extremes of pH and dissolved salts, pressure, light, or host resistance. In a challenge experiment a factor is varied in a representative sample of genetic backgrounds, and the expression levels of all genes are measured in treatment and control with microarrays. For each gene we record whether expression goes up or down, and by how much, in response to the factor. By comparing responses to the different factors at appropriate developmental stages and in appropriate tissues and organs, we can then measure conflicts in the expression needed to achieve two or more functions simultaneously. Such a data set has already been gathered for yeast (Gasch et al. 2000), but the genomic conflicts over whole-organism functions have not yet been analyzed.

The case of reproduction and pathogen attack illustrates this idea. We measure one pattern of whole-genome expression for response to reproduction and another for response to pathogen attack. If the organism were not reproducing, it could defend itself better against pathogen attack, and if it were not under pathogen attack, it could reproduce better. The deviation from the gene expression pattern appropriate to reproduction that we measure when the organism is under pathogen attack while reproducing measures how much it trades off reproductive performance for pathogen resistance. Similarly, the deviation from the gene expression pattern appropriate to pathogen resistance that we measure when the organism is reproducing measures how much it trades off disease resistance in order to reproduce better. Thus, we describe the classical trade-off, or antagonistic pleiotropy, between reproduction and survival in terms of conflicts between the two over whole-genome patterns of gene expression.

If the situation were that simple, the research program would be relatively straightforward, if challenging. The situation is, however, a bit complicated.

The Ambiguous Consequences of Y-Allocation Patterns

Trade-offs have been measured on populations as negative correlated responses to selection. Such responses result from variation in the genetically determined component of allocation patterns among individuals in the population. Within a single individual, one can represent a physiological trade-off as a Y-allocation pattern (van Noordwijk and de Jong 1986; Zera and Harshman 2001). Energy flows up through the base and is allocated into the arms of the Y, with each arm representing a separate physiological function. Arm 1 could get 80% and arm 2 only 20%, for example, which suggests a physiological response favoring function 1 over function 2. How might that be genetically controlled and implemented?

The genes directly responsible for the allocation decision at the fulcrum of the Y should show an expression conflict between the two functions in challenge experiments. That is not, however, necessarily the case for the downstream genes along each of the two arms. In one single-factor challenge, the genes corresponding to arm 1 could be on and those in arm 2 off. Similarly, in a second single-factor challenge, the genes corresponding to arm 1 could be off and those to arm 2 on. If control genes at the fulcrum were in a small minority and genes along the arms dominated the expression response, we would get something like case *A* in figure 2. The lack of conflict in gene expression in the single-factor experiments would then predict no trade-off when one really does exist. A two-factor challenge should, however, provide the remedy, and it would predict a trade-off in this case. Under a double challenge, the expression state responsible for allocation at the fulcrum should be intermediate, and genes along both arms of the Y should be activated and show expression patterns that differ from those in the single-factor experiments.

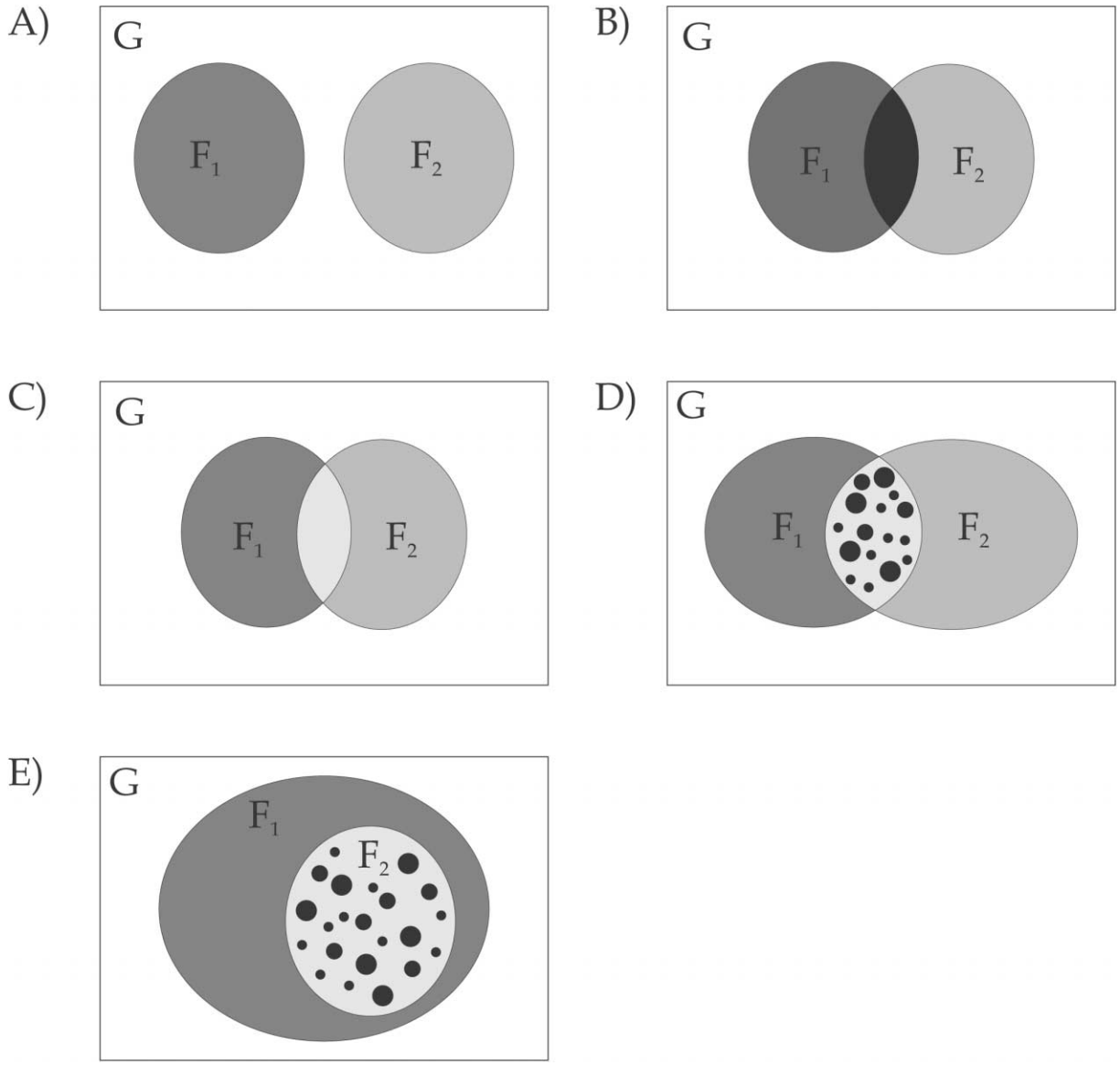

Figure 2: Five types of expression patterns in response to two challenges. *G* is the set of genes whose expression is measured, now often the whole genome. F_1 is the set of genes whose expression changes in response to factor 1, relative to a control. Similarly, F_2 is the set of genes whose expression changes in response to factor 2, relative to a control. Gray in the zone of overlap indicates disagreement over the gene expression needed to respond to a challenge, which suggests genomic conflict over gene expression. White indicates agreement over the gene expression needed to respond to a challenge, which suggests no conflict over gene expression. See text for a discussion of the implications of each of the five cases.

Visualizing Trade-offs as Gene Expression Interactions

Five simple scenarios (fig. 2) characterize the range of genomic responses that one might observe when an organism is subjected to a pair of physiological challenges. *G* represents the set of genes whose expression is measured: the whole genome. F_1 represents the genes recruited to meet the first physiological challenge, and F_2 represents the set of genes recruited to meet the second physiological challenge.

Case A. The sets of genes necessary to mount a response to either challenge are nonoverlapping; therefore, the system can respond effectively to a simultaneous challenge. There is zero genetic correlation between functions.

Case B. Sets F_1 and F_2 overlap. The expression states required to mount a response are dissimilar in their in-

tersection, which results in conflict over gene expression that has traditionally been measured as antagonistic pleiotropy or a negative genetic correlation. Both physiological responses exhibit unique genetic variance.

Case C. Sets F_1 and F_2 overlap. The expression states required to mount a response are similar in their intersection, which results in agreement over gene expression that has traditionally been measured as positive genetic correlation. Both physiological responses exhibit unique variance.

Case D. Sets F_1 and F_2 overlap with a mix of similar and dissimilar expression states. The net effect (i.e., agreement vs. conflict) can be represented by the ratio (possibly weighted) of similar to dissimilar expression states. The genetic correlation is either positive or negative depending on the net effect. Both physiological responses exhibit unique variance.

Case E. Set F_2 is a subset of F_1. The genomic response to challenge 2 is a subset of the genomic response to challenge 1. The genetic correlation is either positive or negative depending on the net effect. The physiological response to challenge 2 exhibits no unique variance.

These diagrams suggest several points. First, the genetic capacity for independent variation of physiological response 1 is proportional to $|F_1 \cap F_2^c|$. Similarly, the genetic capacity for independent variation of response 2 is proportional to $|F_1^c \cap F_2|$.

Second, case *E* stands out as having no unique genetic variance for the second factor. Thus, functional genomic measurements of gene expression patterns could interact with traditional quantitative genetic measures to discriminate hypotheses.

Third, these figures suggest a way to test the approach using data from the challenge experiments. If two functions are in conflict over gene expression patterns, their nonadditive effects on fitness components should increase in the dual-challenge experiments.

Fourth, the sets are fuzzy because of Type I and Type II errors. The areas positively identified contain some false positives, usually 5%; the borders exclude false negatives, sometimes many of them. Pilot data suggest that many of the genes not in an area of overlap might really belong in an area of overlap. That issue—the fuzziness of the set boundaries—suggests two procedures to check the reliability of the set classification: first, construct the sets with Type I error = 0.05, 0.10, or 0.20 to see whether any qualitative conclusions change as increasing numbers of genes are included in the area of overlap; second, forget the sets and use quantitative measures defined on the whole genome (one could also apply such quantitative measures just to members of an overlap set).

Fifth, the sets suggest qualitative measures of conflict. Let $f_1 = |F_1|$ and $f_2 = |F_2|$ be the number of genes whose expression changes from the control in each of the two single-challenge experiments. Let O (for overlap) be the total number of genes whose expression changes in both single-challenge experiments. Overlap O is measured by the intersection of F_1 and F_2 ($O = |F_1 \cap F_2|$). Let O_a be the number in which the change in expression agrees between the two functions, and let O_c be the number in which the change in expression conflicts between the two functions (thus $O = O_a + O_c$). One conflict measure would then be O_c/O, the proportion of coexpressed genes that are in conflict. Another would be $O_c/(f_1 + f_2 - O)$, the ratio of genes in conflict to genes expressed. Only experiments can decide which one better predicts negative correlated responses to selection.

Testing the New Definition

The next step is to test the conflict definition of trade-off. To do so, we must have measured some strong conflicts and some weak conflicts over gene expression. We can then ask, does the degree of conflict over gene expression accurately predict the correlated responses to selection in the artificial selection experiments that have classically been used to measure trade-offs and antagonistic pleiotropy? For both a case of strong conflict and a case of weak conflict, we would perform artificial selection. We would use a control that does not select for resistance to either factor, a treatment in which resistance to the first factor is selected to increase, and a treatment in which resistance to the second factor is selected to increase. If in the control there is no correlated response and in the treatments we get a strong correlated response when conflict is strong and a weak correlated response when conflict is weak, then we have validated the definition of both trade-offs and antagonistic pleiotropies as conflicts in gene expression.

The Costs of Compensatory Evolution

This combination of functional genomics with artificial selection addresses another important question. If we impose a double challenge in which resistance to both factors is selected to increase, we ask the organisms to solve two problems at once. The experiment isolates these two factors from the many challenges that forced other compromises on the organisms in the wild. Can evolution exploit this isolation to reduce the costs paid for the compromise? If it does reduce the conflict between the two functions isolated in the experiment, does the conflict between those two functions and a third function increase? And does the increase in conflict with other functions measure the costs that would have to be paid if we restored the species to the full complexity of its original habitat and asked it to solve many problems at once? Is that what happens when

domestic species go wild, forming feral populations? Some of these questions can be answered by measuring gene expression patterns every few generations during the response to artificial selection.

Some General Questions about Evolution Stimulated by Genomics

Functional genomic studies allow us to address long-standing issues from a new perspective; they also provide the opportunity to investigate a class of problems that were previously unexplored or considered largely inaccessible to experimental investigation. We highlight some of these issues below. Where relevant, we make note of recent studies that bear on these problems.

Evolution of Gene Expression

How much variation in gene expression is there within and between populations? How much of evolutionary change can be ascribed to transcribed DNA and how much to changes in cis-regulatory networks, transcription factors, and transcription factor binding sites (cf. Stern 2000)? Does microevolution result primarily from changes in the DNA sequences of "structural" genes and macroevolution primarily from changes in gene regulation? Or are microevolution and macroevolution a mixture of both? What changes in the transcriptome occur under directional artificial selection? What changes in the transcriptome occur under plastic change within a genotype? What is the relationship between the two? Can such studies be used to settle the discussion about whether there are any genes for plasticity itself and whether it is helpful to consider the state of expression of a trait in a series of environments as a series of independent traits?

A number of recent studies provide some insights into such questions. For example, Oleksiak et al. (2002) studied variation in the expression of 907 genes within and among natural populations of the teleost fish *Fundulus*. The authors were able to show that significant amounts of variation in gene expression exist both within and between populations. In addition, they presented evidence consistent with the hypothesis that differences in gene expression between northern and southern populations are the result of natural selection associated with the demands of thermal metabolism (Oleksiak et al. 2002). In a study with somewhat different goals, Rifkin et al. (2003) studied the development of gene expression in three closely related *Drosophila* species: *D. simulans*, *D. yakuba*, and *D. melanogaster*. Their results are consistent with but not yet demonstrative of conservation of the expression of regulatory genes and more rapid change of structural genes, at least at the level of closely related species.

Comparative analyses such as these begin to provide a basis for answering questions about the evolution of the transcriptome. We expect that similar proteomic studies will further enrich our understanding of the temporal and spatial roles played by particular genes and the relationship of such patterns to microevolutionary and macroevolutionary differences among populations and species.

Evolutionary Physiology

What are the whole-organism functions defined by transcriptional regulation of all the genes in the genome under challenge by important ecological factors such as temperature, starvation, and pathogen attack?

Pletcher et al. (2002) recently completed a study of age-related changes in the transcriptome in well-fed and starving *Drosophila*. The motivation of the study was the observation that of all phenotypic interventions that extend life span, caloric restriction is the one that produces large effects most reliably and in several distinct types of organisms (yeast, nematodes, flies, mice, and humans). Therefore, the study of transcriptional changes in starved flies could yield insight into general mechanisms capable of extending life span.

The whole-genome transcript profiles contained a statistically powerful genetic signature of normal aging, with approximately 3,000 genes (nearly 23% of the roughly 13,600 genes) changing in expression pattern with age. The extension of life span by caloric restriction was accompanied by a slowing of the progression of normal age-related changes in transcript levels. They found no evidence that age-dependent changes in transcription were localized to specific regions of the genome and no support for widespread disregulation of gene expression with age.

The caloric restriction study raises an issue that will accompany genomic research for a long time: results couched in terms of changes in expression profiles of thousands of genes do not help much unless they can be chunked into workable units in a meaningful fashion. It only makes sense to abandon the one-gene-at-a-time strategy for a genomic approach if the genomic approach yields information that can be thought about clearly and brought to bear on other problems in a precisely definable and concrete manner. The appropriate chunking of gene expression data into workable units is a major genomic challenge. Ideas developed in the context of evolutionary biology may provide useful ways to attack this problem.

Genotype-Phenotype Mapping

The relationship between genotypes (G) and phenotypes (P) and the attempt to understand the myriad genetic and environmental interactions that determine the $G \rightarrow P$

mapping is a fundamental problem for all of biology. Much of the progress in this area has been in the development of theoretical and mathematical treatments that relate variation in underlying genetic or developmental parameters to variation in phenotypic traits (e.g., Rice 1998, 2002). Quantitative trait loci (QTL) studies and related approaches (e.g., Cheverud et al. 1996) have provided some experimental insights into this problem but primarily in terms of identifying genes that are likely parameters of the G → P mapping as opposed to a functional description of the mapping itself. Functional genomic studies hold forth the promise, as yet unrealized, that we may be able to define the parameter space as well as estimate the functional forms of at least some G → P mappings. A recent study that points in this direction is the work of Huang et al. (2002), who were able to correlate the expression of particular genes with physiological and physical parameters of the rat circulatory system. Their conclusions have the flavor of QTL analyses (i.e., identifying candidate genes that affect the phenotype of interest). However, the nature of the gene expression data is such that through a combination of functional data analysis (Ramsay and Silverman 1997), experimental perturbation, and artificial selection in a model system, one ought to be able to begin to estimate the form of G → P mappings for these traits and test the predictions stemming from mathematical models such as those of Rice (1998, 2002).

Evolutionary Systems Biology

Systems biology is an emerging approach that focuses on the study of biological processes and phenomena at the level of whole systems rather than as a set of isolated parts (e.g., Kitano 2001). While the term "systems biology" is relatively new, the goal of developing holistic approaches to understanding biological systems has a long history. Now high-throughput genomewide transcriptional and proteomic assays make it possible to undertake quantitative studies of biological processes in fundamentally new ways. For example, figure 3 depicts a genetic regulatory network based on 1,000 yeast genes. This network was constructed by estimating conditional independence relationships for pairs of genes on the basis of genomewide transcriptional assays. The nodes of the network represent genes, and the edges indicate which pairs of genes have robust conditional interactions (P. Magwene, unpublished data). Such networks have many potential uses. For example, they can be used as a sophisticated means of associating genes of unknown function with previously annotated genes. In a complementary fashion, such networks can be used to classify sets of functions whose genetic bases overlap. From the viewpoint of organismal and evolutionary biology, these networks suggest that the following questions are ripe for investigation: How do regulatory networks change through development, and how do they differ among populations of closely related species? How do regulatory networks evolve? Do genetic regulatory networks exhibit modularity, and can this modularity be related to our understanding of homology (e.g., Wagner 2001)?

Another example of the type of systems perspective that is facilitated by functional genomics data is illustrated in figure 4, in which the age-dependent expression of about 4,000 *Drosophila* genes has been compressed to a single line snaking through a three-dimensional space that captures most of the variation across the genome in genes whose expression changes significantly with age (J. Kim and S. Rifkin, unpublished data). Age varies along the line; the portions of the line corresponding to egg, larval instars, pupa, and imago are indicated. In some as yet undecoded and clumsy sense, that line captures the genetic essence of *Drosophila* life history: how to build a fly that survives and reproduces, then ages and dies. Such a statistical description of an age-specific but tissue-, organ-, and trait-unspecific whole organism sample of the transcriptome may, at this stage, be more motivational than practical, but motivation has its uses.

How Will Evolutionary Biology Affect Functional Genomics?

Throughout this article we have argued that functional genomics is likely to have a large impact on a number of fundamental concepts in evolutionary biology. Similarly, we envision a reciprocal impact on functional genomics from evolutionary biology. One of the major areas in which this is likely to be evident is in the adoption of comparative methods and the incorporation of "population thinking" (Mayr 1975; Templeton 1999).

Templeton (1999) argues cogently that a failure to explicitly incorporate information about human genetic diversity into the early goals of the Human Genome Project was a major stumbling block in the path of one of the project's stated goals: to serve as a resource for studying human diseases. The goals of the Human Genome Project were later amended to include such information, and Templeton (1999) provides a number of examples that illustrate how adopting the viewpoint of "population thinking" is critical for studying phenotypes with a complex genetic architecture. Comparative methods allow us to exploit the principle that the "most meaningful contrasts are between evolutionary neighbors," and in doing so we are able to "concentrate statistical power upon the most relevant comparisons" (Templeton 1999). Analytical techniques based on the tenets of comparative biology already play an important role in genomics, with BLAST (Altschul et al.

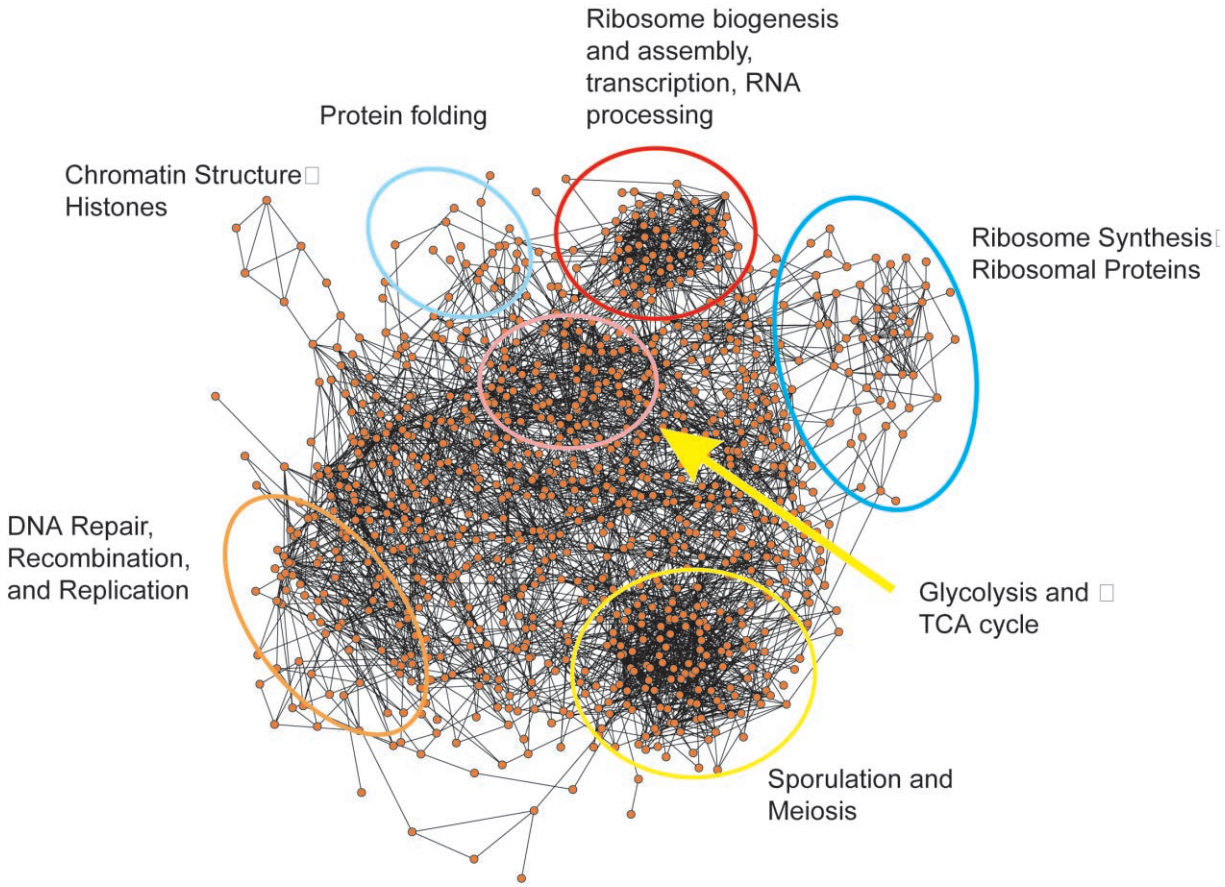

Figure 3: Genetic regulatory network estimated from microarray measurements of gene expression for the yeast *Saccharomyces cerevisiae* (P. Magwene, unpublished data). Several functionally relevant clusters of genes are highlighted in the figure. The study of how such networks change through development and over evolution is among the new class of problems illuminated by functional genomic studies.

1997) being among the best-known examples. We predict that analytical and experimental tools designed to exploit natural variation in the magnitude (e.g., Cowles et al. 2002) and timing (e.g., Kim et al. 1999) of gene expression will become important pieces of the functional genomics toolbox.

Another example of the practical import of population thinking for functional genomics can be found in a consideration of the concept of modularity. A modular system is one that is decomposable into a set of modules (subsystems), each of which is independent or semi-independent from other such modules. There are strong interactions among the parts that make up a module but only weak interactions between components of different modules. Modularity is primarily quantified through the study of interspecific or intraspecific patterns of covariation (Magwene 2001). How modular systems evolve and how modularity relates to concepts such as robustness, canalization, and evolvability are the focus of a number of recent studies (e.g., Mezey et al. 2000). From a practical point of view, modularity is a useful concept for decomposing a complex system into a set of smaller subsystems, each of which can be studied in isolation. If we can divide up a complex system in a manner consistent with the true patterns of semi-independence in the system, then little or no information about the behavior of the system as a whole is lost when the modules are studied as separate components. Modularity therefore provides an important conceptual and experimental handle for dealing with the complications introduced by the study of whole genomes. Quantitative methods for characterizing modularity will be a vital tool for breaking down complex gene expression data into "workable units."

Conclusion

There are good reasons to have functional genomicists down the hall from and interacting with evolutionary ge-

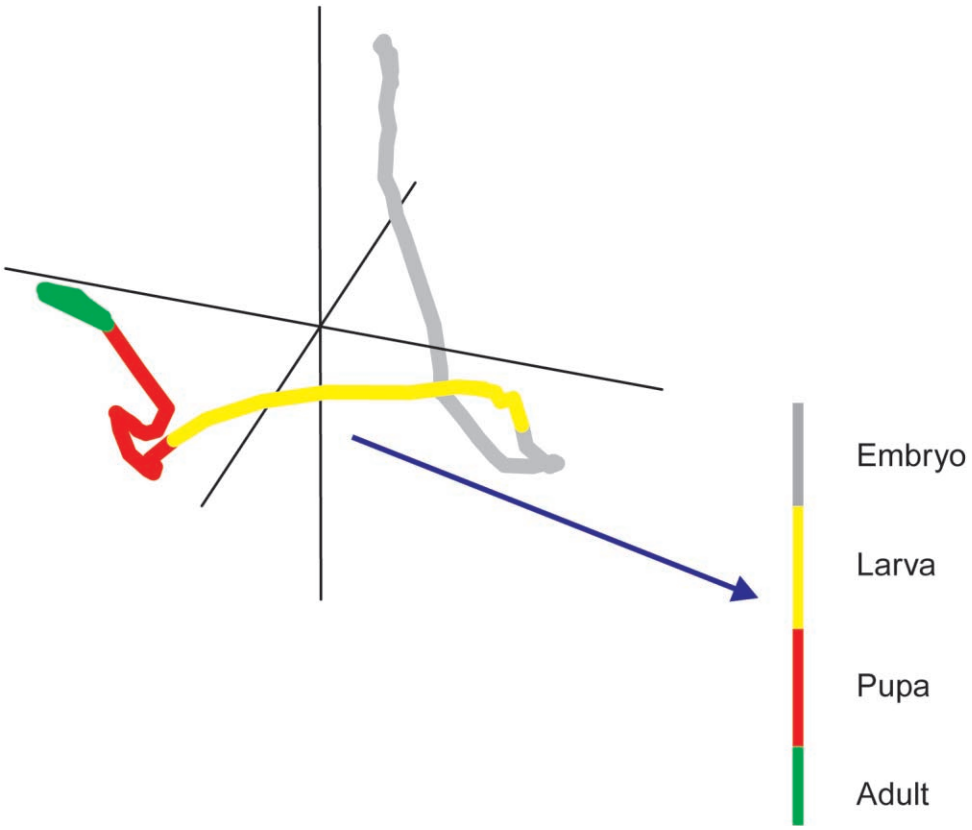

Figure 4: Single line describing the track of the expression patterns of about 4,000 genes in developing *Drosophila* through a three-dimensional space that describes most of the variation in the data (J. Kim and S. Rifkin, unpublished manuscript).

neticists, morphologists, physiologists, and evolutionary and behavioral ecologists. At the moment, genomics is intensely involved in the descriptive natural history of the genome, the transcriptome, and the proteome. Once it has helped to deliver a solid description of underlying mechanisms, some core evolutionary concepts may be heavily modified or replaced by more concrete ones. The areas in which that is likely to happen are indicated by a few of the big questions stimulated by genomics: Does regulatory change correspond to macroevolution and transcriptional change to microevolution? How do transcriptomes and proteomes change under directional artificial selection? under speciation? How much of evolutionary change can we perceive at the level of cells, and how much only by taking into consideration the integration of whole organisms? These questions, among the many that can now be asked, suggest the revolutionary nature of these data. Most such questions could not be investigated experimentally as little as five years ago; now they are rapidly becoming part of the art of the possible.

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