Genetica **112–113:** 45–58, 2001.

Toward a new synthesis: population genetics and evolutionary developmental biology

Norman A. Johnson & Adam H. Porter
Department of Entomology and Program in Organismic and Evolutionary Biology, 102 Fernald Hall, University of Massachusetts, Amherst, MA 01003, USA

Key words: developmental genetics, evolution of development, **G** matrices, genetic co-option, genetic pathways, mutation effective size, population genetics, quantitative genetics, speciation

Abstract

Despite the recent synthesis of developmental genetics and evolutionary biology, current theories of adaptation are still strictly phenomenological and do not yet consider the implications of how phenotypes are constructed from genotypes. Given the ubiquity of regulatory genetic pathways in developmental processes, we contend that study of the population genetics of these pathways should become a major research program. We discuss the role divergence in regulatory developmental genetic pathways may play in speciation, focusing on our theoretical and computational investigations. We also discuss the population genetics of molecular co-option, arguing that mutations of large effect are not needed for co-option. We offer a prospectus for future research, arguing for a new synthesis of the population genetics of development.

Introduction

Biologists studying and predicting micro-evolutionary change generally focus on variation within populations. One major sub-field of micro-evolutionary studies is population genetics, which in the strictest sense involves tracking changes of frequencies of alleles and genotypes within and among populations across time and space (Futyuma, 1998). Quantitative genetics, another sub-field of micro-evolution, by contrast is a "statistical branch of genetics based upon fundamental Mendelian principles extended to a polygenic (multilocus) characters... phrased in terms of phenotypic means and variances" (Lynch & Walsh, 1998, p. 5). The line between population and quantitative genetics, especially with the prevalence of quantitative trait loci (QTL) studies today, is blurred. In this perspective, we will often use these terms interchangeably.

Population and quantitative geneticists generally have neglected the details of how phenotypes are constructed from genotypes; typically this has been the province of developmental geneticists. As a result, scant attention has been paid to the impact of micro-evolutionary forces and processes on regulatory and developmental systems. Current theories of adaptation remain strictly phenomenological and do not yet incorporate molecular genetic principles. We argue here for a synthesis of population genetics and developmental biology, distinct from the current synthesis of evolution and development with its emphasis on phylogenetic history and the new comparative method. The synthesis of population genetics and development would form the basis of a micro-evolutionary theory of adaptation rooted in knowledge of how phenotypes are constructed from genotypes.

We begin in Section I with a discussion of historical and conceptual foundations, describing the syntheses that have led to developmental genetics and evolutionary developmental biology. In Section II, we discuss how the field of evolutionary quantitative genetics can benefit by opening up the black box of the **G** (variance–covariance) matrix of traits via more detailed examinations of the developmental processes linking genotype and phenotype. In Section III, we discuss how changes in regulatory genes need not imply macro-mutations. We affirm the idea that multiple

mutations of relatively small effect at a few loci may often be responsible for large evolutionary changes. In Section IV, we argue that macro-mutations are not needed to explain the co-option of new genes into regulatory genetic pathways. We also argue that the population genetics of how new regulatory interactions are subsumed into existing developmental pathways is a fundamental problem to solve in this new synthesis.

The next and largest section (V) is a discussion of the role that divergence in regulatory developmental genetic pathways may play in speciation. We summarize the results of our modeling the impact of micro-evolutionary forces on linear regulatory pathways, showing that divergence in regulatory pathways is conducive for the evolution of reproductive isolation (Johnson & Porter, 2000). In addition, we discuss how our results from simple, linear pathways can be extended to more complex, branched pathways. We conclude with a prospectus for future research, highlighting our vision of the new synthesis.

I. Historical and conceptual foundations

For much of the 20th century, genetics and development were separate disciplines with scant cross-communication. Just how disconnected these disciplines seemed can be gleamed from Boris Ephrussi's words below. Recounting a discussion he had with Thomas Hunt Morgan in 1934 about Morgan's new book, Ephrussi (1958, quoted in Gilbert, 1988) stated:

> "I said (to Morgan) I found the book very interesting, but I thought that the title was misleading because he did not try to bridge the gap between embryology and genetics as he had promised in the title. Morgan looked at me with a smile and said, 'You think the title is misleading! What is the title? 'Embryology and Genetics', I said. 'Well', he asked, 'is not there some embryology and some genetics?' This shows how polarized I was on the gap between embryology and genetics, and how anxiously I was waiting for somebody to bridge it." (see also Kohler, 1994)

Beginning in the 1970's, this gap was bridged. Genetics and embryology, previously separate disciplines, were synthesized into the very successful field now known as developmental genetics. What is the role of genes in development? With rare exceptions, all of the cells of a metazoan have essentially the same genes. In each cell type, however, only a certain subset of the genome is expressed (Davidson, 1986; Gilbert, 1997; Levin, 1997). Which genes are expressed and the level of expression vary across cell type. Thus, development consists of the differential turning on and off of genes in cell types at appropriate locations and times.

Understanding the role of genes in development first required understanding how genes are expressed and how gene expression is regulated. We now know the general principles underlying eukaryotic gene regulation. Much regulation occurs at the level of transcription and is mediated by protein-DNA and protein–protein interactions. Specifically, proteins (called 'transcription factors' or TFs) bind to *cis*-regulatory elements which are DNA sequences nearby the coding regions of genes. This binding affects the transcriptional machinery and thus can alter the level of transcription of the gene product. The proteins that can interact with regulatory modules are remarkably diverse (Aranone & Davidson, 1997). Protein–protein interactions also play an important role in many pathways, particularly in those signaling pathways involving intercellular communication (e.g., the *Notch* cascade; Munn & Kopan, 2000). Several other classes of regulatory interactions are known. Obviously, allelic variants can have different regulatory efficacy in these interactions.

One early success story of developmental genetics was the determination of the genetic mechanisms underlying segmentation in *Drosophila melanogaster* (Lewis, 1978; Nusselein-Volhard & Weischaus, 1980). Cascades of genes establish the general pattern, with segmentation being determined by the sequential expression of some genes and suppression of other genes in this cascade (See Figure 1). The products of these genes are transcription factors (TFs) which regulate the expression of genes further downstream in the cascade. Toward the end of the cascades are the genes responsible for establishing segment identity, the homeotic genes, and the targets of their proteins (Lewis, 1978; Bender et al., 1983). Cascades are not unique to the determination of segmentation; indeed, they are common features of developmental and physiological processes. One notable class is the sex-determination pathways (e.g., Schutt & Nothinger, 2000 and references within), where a decision (male v.s. female) is made and amplified via a signaling cascade, often involving differential splicing of a primary RNA transcript (a protein-RNA interaction).

Findings from this developmental genetics synthesis also paved the way for a synthesis between

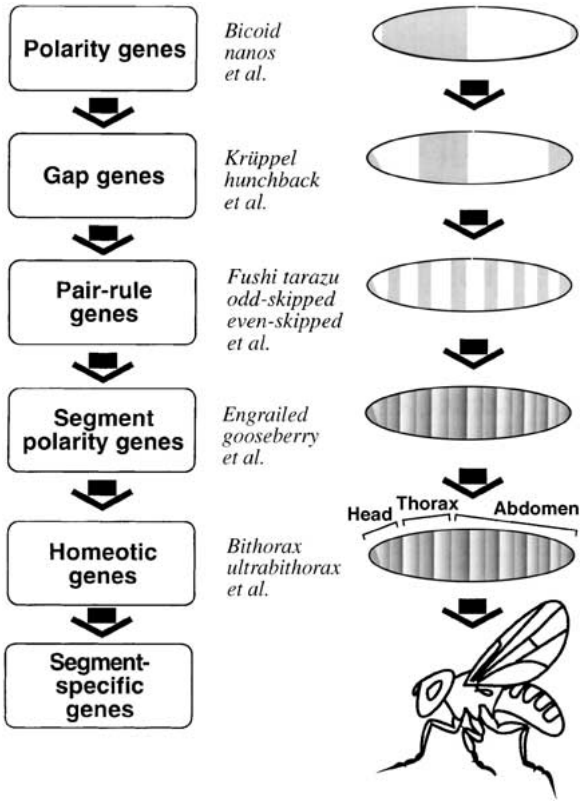

Figure 1. Developmental genetic cascade, with earlier genes affecting the expression of later genes during embryogenesis. Polarity genes establish anterior–posterior polarity of the embryo and regulate expression of gap genes. These define major body regions and regulate pair-rule genes, which define the segment boundaries. The pair-rule genes regulate expression of the segment-polarity genes that determine the anterior–posterior polarity of individual segments. These then regulate expression of the homeotic genes, which define the identities of the individual segments. The homeotic genes then regulate development of segment-specific structures.

aspects of developmental biology and evolutionary biology. Darwin was very interested in embryology and indeed, he presented comparative embryology as one central argument for his theory (Darwin, 1859; Jones, 1999). During the first half of the 20th century, various biologists have attempted to synthesize development and evolution (e.g., Garstang, 1922; Goldschmidt, 1940; Thompson, 1942). However, these attempts mostly failed due to a lack of a common framework between researchers of the different disciplines.

The discovery of the homeobox, a striking feature of genes involved in transcription regulation, allowed for this common framework. First found in the homeotic genes, the homeobox is a protein-encoding consensus sequence of 180 nucleotides found in many transcription factors. Homeoboxes encode amino-acid sequences (homeodomains) that recognize and bind to specific DNA sequences. The homeoboxes are extremely similar across the different genes that possess them; pair-wise comparisons often show 80–90% sequence similarity (McGinnis et al., 1984b; Scott & Weiner, 1984; Lewin, 1997). Homeoboxes turned out to be not just an oddity of *Drosophila melanogaster*, or insects, or even segmented animals. Instead, they have been found in virtually all metazoans (McGinnis et al., 1984a; Raff, 1996; Gilbert, 1997; Pederson et al., 2000). Not only are the sequences of the genes conserved across widely diverged groups, so are their functions. Finding the conservation of homeoboxes was an important impetus in the creation of the synthesis, evolutionary developmental biology (a.k.a. evodevo), between developmental genetics and the phylogenetic subdiscipline of evolutionary biology.

Several other advances occurred during the same period of time, promoting the evodevo synthesis. The comparative method, a tool of developmental and functional biologists since Weissman, had matured to explicitly incorporate a phylogenetic perspective. During the 1980's and 1990's, comparative biologists have developed increasingly sophisticated methods to study character evolution that take into account the lineage history of the taxa involved (Felsenstein, 1985; McPeek, 1995; Martins, 1996). Systematics has undergone a similar revolution with theoretical advances in the testing of phylogenetic hypotheses, a tremendous increase in computational capacity, and the generation of a wealth of molecular sequence and morphological data (Hillis, Moritz & Mable, 1996 and references within).

One surprise emerging from these evodevo studies was the deep conservation of genetic structure and function. As Palopoli and Patel (1996, p. 502) note, "animals that look nothing like each other develop by using much the same basic 'tool-kit' of molecules and often in the same ways". A particularly striking example of this conservation across vastly different lineages is the *eyeless/Pax-6* gene, a putative master switch for eye development (Quiring et al., 1994; Halder, Callaerts & Gehring, 1995). Halder, Callaerts and Gehring (1995) showed that ectopic expression of the mammalian homologue of the *Drosophila* eyeless gene can induce compound (fly-type) eyes to grow almost anywhere on a fly. This is despite the independent evolution of the structures of fly and mammalian eyes. As emphasized by Gerhart and Kirschner (1997), studies like Halder, Callaerts and Gehring (1995)

focus more on the striking degree of conservation of genetic systems across ancient lineages than upon divergence (but see Sucena & Stern, 2000).

A wide gulf still divides the approaches of the contemporary evodevo and population genetic schools. Most population genetics research focuses on the evolutionary forces (natural selection, random genetic drift, mutation, and gene flow) influencing the frequencies of alleles or polygenic phenotypes over a fairly short period of time. In contrast, evolutionary studies of development typically focus on differences in the expression of rather conserved genes between fairly distant taxa (e.g., beetles v.s. flies), questions that are better addressed using phylogenetic and comparative methods than using the machinery of population genetic theory (see also Gilbert, 2000). The evodevo studies emphasize what happened deep in the history of life but not how these developmental systems continue to evolve. To accomplish the latter, we need to incorporate the principles of population genetics into the study of regulatory pathways, and vice versa. We agree with Gilbert, Opitz and Raff's (1996) call to arms: "The population genetics of regulatory genes and their possible combinations within fields should become a major new research program" (p. 368). Below we suggest how aspects of population and quantitative genetic theory can be integrated into the evodevo synthesis.

II. Quantitative genetics and the genotype–phenotype map

Biologists from diverse disciplines, including evolution, paleontology, morphology, and development, have long recognized that the relationship between genotype and phenotype can be very complex (e.g., Wright, 1968; Lewontin, 1974; Arnold, 1983; Feder & Watt, 1992; Conway Morris, 2000). In contrast, quantitative genetic approaches traditionally neglect the underlying genetic basis of traits in favor of an analytically more tractable statistical description. For instance, consider the breeders' equation: $\Delta\mathbf{Z} = \mathbf{G}\boldsymbol{\beta}$. This mainstay of quantitative genetics shows how one can predict selection-induced changes in one trait when its expression is correlated with the expression of other traits (Falconer & MacKay, 1996; Lynch & Walsh, 1998). The elements of $\Delta\mathbf{Z}$ represent the changes in each trait over one generation, $\boldsymbol{\beta}$ holds the selection gradients (i.e., phenotype v.s. fitness relationships) of each trait, and $\mathbf{G}$ is the matrix of the genetic variances and covariances among the traits (see Figure 2). All of the unknown genetic and physiological details about how traits correlate are subsumed into the $\mathbf{G}$ matrix.

(a) $$\begin{bmatrix}\Delta Z_1\\ \Delta Z_2\end{bmatrix} = \begin{bmatrix}\mathrm{Var}_{(G)1} & \mathrm{Cov}_{(G)1,2}\\ \mathrm{Cov}_{(G)1,2} & \mathrm{Var}_{(G)2}\end{bmatrix}\begin{bmatrix}B_1\\ B_2\end{bmatrix}$$

(b) $$\begin{bmatrix}\Delta Z_1\\ \Delta Z_2\end{bmatrix} = \begin{bmatrix}2.0 & -0.5\\ -0.5 & 1.0\end{bmatrix}\begin{bmatrix}0.2\\ 0.1\end{bmatrix}$$

(c) $$\begin{bmatrix}\Delta Z_1\\ \Delta Z_2\end{bmatrix} = \begin{bmatrix}(2.0)(0.2) + (-0.5)(0.1)\\ (-0.5)(0.2) + (1.0)(0.1)\end{bmatrix}$$

(d) $$\begin{bmatrix}\Delta Z_1\\ \Delta Z_2\end{bmatrix} = \begin{bmatrix}0.35\\ -0.15\end{bmatrix}$$

Figure 2. The breeder's equation $\Delta\mathbf{Z} = \mathbf{GB}$ is a statistical description of the evolutionary interdependence of traits that share common genetic bases. It presumes that each trait varies in the population, and that some of the variation is due to allelic differences. $\Delta\mathbf{Z}$ is the evolutionary change seen in the average measured for each trait. $\mathbf{B}$ is the selection gradient; for example, a positive value of B for a trait indicates that larger values are favored by selection. $\mathbf{G}$ is a matrix of the genetic variances of each trait and the genetic covariances among them. The genetic variance is the proportion of the total variation that is caused by underlying genetic variation among individuals. A positive covariance means that individuals larger than average for one trait are also likely to be larger than average for the other trait. Here is a telling example worked out for a pair of traits. (a) $\Delta\mathbf{Z} = \mathbf{GB}$ in expanded matrix form. ΔZ_1 and ΔZ_2 are the evolutionary changes expected in traits 1 and 2. B_1 and B_2 are the extents that selection favors larger values of the two traits. In the matrix $\mathbf{G}$, $\mathrm{Var}_{(G)1}$ and $\mathrm{Var}_{(G)2}$ are the genetic variances for traits 1 and 2, and $\mathrm{Cov}_{(G)1,2}$ is the genetic covariance between the traits. (b) Numerical example, where there is a negative covariance between the traits, and different strengths of selection. (c) Multiplying out the matrix gives (d) the extent of evolutionary change in each of the traits. Notice that even though selection favored an increase in trait 2 (i.e., B_2 is positive), its value decreased ($\Delta Z_2 < 0$) because of the negative covariance with trait 1. Therefore, the equation $\Delta\mathbf{Z} = \mathbf{GB}$ says that the evolutionary change for each trait ($\Delta\mathbf{Z}$) depends not only on selection on ($\mathbf{B}$) and variation in each trait (the diagonal, variance elements of $\mathbf{G}$), but also on the extent that the traits are genetically interdependent (the off-diagonal, covariance elements of $\mathbf{G}$).

Quantitative geneticists interested in making long-range predictions of selection responses have been very interested in the extent to which $\mathbf{G}$ matrices are stable (Arnold & Phillips, 1999; Phillips & Arnold, 1999; Roff & Mousseau, 1999; Roff, 2000; Arnold, Pfrender & Jones, 2001; Phillips, Whitlock & Fowler, 2001). If stable, the breeders' equation would be

useful for long-term evolutionary predictions. If not, then predictions about trait evolution would depend upon how these matrices evolve (see also Barton & Turelli, 1989; Arnold, Pfrender & Jones, 2001). In fact, both theoretical and empirical evidence clearly demonstrate the pliability of **G** matrices over evolutionary time. Empirical quantitative geneticists have long recognized the difficulty in predicting how a second, genetically correlated trait will evolve when artificial selection is applied to a targeted trait. The difficulty arises because a given genetic correlation can be produced by different underlying genetic interactions, and because these interactions themselves evolve, by selection and sampling drift, during the course of these experiments (Gromko, 1995; Lascoux, 1997). Population bottlenecks (Bryant et al., 1986; Goodnight, 1987) and inbreeding (Phillips, Whitlock & Fowler, 2001; see also Pray & Goodnight, 1995) also lead to substantial evolution of **G**-matrix components, probably by fixing, or substantially changing frequencies of, genotypes at loci that underlie the traits. In extreme cases, even the signs of the covariances in the **G** matrices change. Given this evolutionary lability, the breeders' equation is too simple to predict long-term evolutionary change (Pigliucci & Schlichting, 1997). Ultimately, these predictive models should be grounded in a mechanistic understanding of how **G**-matrix components evolve.

Recently, several researchers have led efforts to reduce the descriptive mathematics of quantitative genetic theory to a level that includes the effects of underlying developmental genetic processes on phenotypic variation. Rice (1998, 2000) partitions phenotypic variation in a very general way that emphasizes epistatic interactions and the interactions of individual genes with arbitrary environmental components. The outcome is a theoretical framework for interpreting not only phenotypic evolution within a developmentally relevant context, but also the evolutionary pressures affecting the sensitivity of developmental systems to environmental variation. It is therefore a general model of phenotypic evolution that readily encompasses developmental canalization and its complement, phenotypic plasticity. The elements of his model are the unspecified functional relationships of alleles with one another and the environment. A fundamental conclusion is that these underlying functional relationships at the allelic level have to be non-linear for phenotypic plasticity to evolve. Their non-linearity is perhaps to be expected on physiological grounds, as many of these relationships involve complex physiochemical interactions of molecules encoded by these loci.

Hansen and Wagner (2001) take a wholly different tack, but arrive at a similar place. They avoid single-gene (e.g., additive) effects altogether and model only gene interactions, specifically the effects of the substitution of a new allele on the phenotypic contribution made by all other alleles. As new mutants invade, the entire genetic background changes as these new alleles influence the phenotypic effects of extant alleles. The result is a genotype-to-phenotype map with extraordinary evolutionary lability, a far cry from the stable **G** matrix needed to apply the breeders' equation. Wolf et al. (2001) extend a third approach that reduces phenotypes to the contributions of underlying developmental modules, which may in turn interact with one another and the environment, and change temporally, in arbitrary ways.

Each of these approaches includes development in ways that essentially retain the descriptive, linear-model qualities of the quantitative-genetic approach. Critical to the dynamics of each is what Wagner (1996) calls the mutation effects matrix: the effect of new alleles on the phenotypic expression of existing alleles. Especially in the Rice (2000) and Hansen and Wagner (2001) models, these allelic effects are partitioned to a level that they can be measured individually in carefully controlled laboratory experiments. Doing so for a very simple developmental system will be an important step for verifying these models. Even when this is done, however, these models do not translate into predictive models of evolutionary change, unless all possible allelic effects, in all possible combinations, have been measured. To make them more predictive, so that allelic effects can be anticipated from first principles, the connection ultimately needs to be made to the underlying molecular and physiological mechanisms that produce them. Mechanisms are needed for representing how different DNA sequences might translate into phenotypic effects, through physiochemical rules that govern the binding among proteins and nucleic acids – part of the research program of proteomics (Boguski, 1999; Fields, 2001). Rice's (2000) model is perhaps the most amenable to the incorporation of these rules, because the functional relationships of how alleles affect one another are left unspecified.

While the ambitious goal of a predictive model of phenotypic evolution is well beyond our current capacity, important limited progress can be made using very simple developmental systems. Population/quantitative genetic studies of regulatory

developmental pathways can address a number of questions about details of the links between genotype, phenotype, and fitness.

(a) How do nucleotide and amino-acid changes affect regulatory binding properties?
(b) How do changes in binding properties affect levels of gene expression?
(c) How do changes in gene expression affect the phenotypes influenced by the gene?
(d) What are the fitnesses of these phenotypic variants?

Questions (a–c) are within the realms of physical and biochemistry, molecular developmental genetics and to some extent, developmental physiology. Question (d) is an ecological-genetics question that can provisionally be addressed by measuring fitness in controlled laboratory environments. Answering these questions, even in very simple developmental systems, will help us understand the developmental and physiological reasons why different traits vary together. This understanding, in turn, will give us the connection we need between evolutionary studies at the level of the gene (and genetic pathways) and the statistical language we use to study the complexity of evolutionary pressures on covarying traits in an ecological setting.

III. Mutation effect size: tinkering with Fisher's microscope

In the 1930's and 1940's, Richard Goldschmidt championed the roles both of developmental processes and of large-scale mutations (a.k.a., macro-mutations) in evolution (Goldschmidt, 1940; Mayr, 1982; Raff, 1996). Perhaps for this reason, regulatory change and macro-mutation have been considered almost synonymous. We believe, however, that the evolutionary significances of regulatory site changes and macro-mutations can be disentangled. Regulatory site changes need not be macro-mutations. Below, we explain this contention and also discuss a new, more-nuanced view on the mutation-effect size debate.

Fisher (1930), using the metaphor of tuning a microscope, proposed that those mutations that contribute to adaptation should have exceedingly small effects. If the microscope is already somewhat in focus, small changes are more likely to lead to further improvement than are larger ones. Much of quantitative genetic theory is based upon the assumption that evolutionary change is due to infinitesimal changes at many loci. This assumption has been pervasive in quantitative genetics both because of Fisher's reasoning and because this genetic architecture is relatively easy to model.

This Fisherian stance has faced several challenges over the last 10 years. Orr and Coyne (1992) reviewed the evidence and found only weak support for the extreme Fisherian view. Since then, various studies have suggested that 'genes of major effect' contribute a substantial fraction of the standing variance for quantitative characters in natural populations of *Drosophila* (MacKay & Langley, 1990; MacKay, 1995, 1996; Lyman & MacKay, 1998). Moreover, only a handful of genetic changes appear responsible for most of the differences in floral characteristics and other morphological changes between two monkeyflower species (Bradshaw et al., 1998; Schemske & Bradshaw, 1999; see also Coyne, 1995). These changes at 'genes of large effect', though smaller than the types of changes Goldschmidt championed, are still much larger than the types of changes Fisher envisioned. Complementing the empirical data, Orr's (1998) model predicts a continuum of allelic effects during adaptation to a new static optimum. Relatively large changes should occur early in the adaptive process, followed by increasing smaller ones. The sizes of mutational effects also depend upon how far populations are from fitness optima and how fast those optima change, ecological assumptions about which little is known.

More recently, a more-nuanced view about mutation-effect size has begun to emerge. The QTLs for species differences uncovered in the monkeyflower study (Bradshaw et al., 1998; Schemeke & Bradshaw, 1999) each consist of large chromosomal regions and encompass very many genes. More recently, several more fine-scale genetic analyses of trait differences have been published. These traits, all in *Drosophila*, include *Adh* activity variation in *D. melanogaster*, bristle number variation also in *D. melanogaster*, and fitness of hybrids between *D. simulans* and *D. mauritina*. In a review of these finer analyses, Phillips (1999) concluded that in these cases, allelic and mutational effects are not synonymous. Rather, "multiple changes within a single locus or small chromosomal region are responsible for natural variation and these changes are often cryptic with respect to what one might traditionally define as an allele. Therefore what we view as an allele in the current context of a population might have been pieced together historically over time from multiple mutations" (Phillips 1999, p. 7). An 'allele'

for a high trait value may differ from an 'allele' for a low trait value by several mutations. Stern (2000) reached similar conclusions and argued that many of these changes are indeed regulatory. Micro- mutations apply to regulatory gene variation just as well as they do to structural gene variation.

Differences between species may also seem, for artifactual reasons, to be due to a few loci of large effect. In the Dobzhansky–Muller model, reproductive isolation accrues as separate populations acquire genetically incompatible alleles at loci that interact epistatically (Johnson, 2000a; see § V). These incompatibilities can arise in small steps, with earlier mutations paving the way for later mutations that magnify the overall incompatibility (Orr, 1995). Late in the process, analysis is likely to erroneously imply that the earlier mutations were of large effect, a result that would be an artifact of the changed genetic background. Indeed, the evolution of the genetic background is likely to introduce complications into any analysis of gene effects between species (Hansen & Wagner, 2001), not just reproductive isolation.

In summary, there are several situations where mutations of small effect are likely to play important roles in the evolution of development. A population genetic approach to variation in regulatory interactions is needed to study their frequency, causes and phenotypic consequences.

IV. Co-opting new genes into regulatory pathways

A fundamental feature of development is that the same genes are used by different genetic pathways, often for different functions at different times and places in the same organism. When examined phylogenetically the effect is magnified: even very different traits in distantly related organisms may be controlled by the same regulatory gene (reviewed in Kauffman, 1993; Raff, 1996; Gerhart & Kirschner, 1997; Nagy, 1998; Conway Morris, 2000). Genetically within an organism, this multiple regulation may be accomplished several ways, but the most common is by having multiple upstream promoter or enhancer sites that influence transcription. Then, different regulatory pathways during development can initiate or block transcription in several different ways (Arnone & Davidson, 1997). It is clear from the differences among phylogenetically distant organisms that adaptive evolution involves not only fine-tuning the expression of regulatory genes that already exist in a developmental pathway, but also the gain and loss of genes in the pathway.

How are genes subsumed, or co-opted, into new pathways? Are macro-mutations needed? We do not think so. We suggest that the evolution of co-option can occur via relatively small mutational steps. The answer will require a deeper empirical understanding of the population genetics of gene regulation, knowledge we do not yet have. However, we can frame the problem.

One way a gene may become incorporated into a pathway is by simply turning off an inhibitory element that binds in its promoter region. In some cases, a simple point mutation in the promoter would be sufficient; in others, this same inhibitor might be required at other times and places in development, whereupon pleiotropic side effects of the mutation would be too costly. Otherwise, genes could be co-opted if they were to evolve a new promoter site sensitive to a regulatory element already available in the pathway. This might not be too difficult. Promoter and enhancer sites are known to occur many hundreds of base pairs upstream from the start codon and often there is considerable so-called junk DNA throughout the upstream region. Enhancer sites can occur in introns or the 3′ untranslated region of a gene. There are likely to be short regions within these regions containing sequences not too different from those needed to bind available transcription factors. It would be very interesting to know the distribution of point mutations, insertions or deletions that could yield a new regulatory interaction, co-opting the gene. Even if there were only weak binding, then provided it had a phenotypic effect and sufficient adaptive advantage, selection could fine-tune it. Gene expression is not simply an on/off phenomenon but instead can be modulated quantitatively, as it is in our models (Johnson & Porter, 2000; also see above). Adding a new inhibitory interaction might work in a similar way.

V. Speciation and the population genetics of development

Speciation often has been considered as both the conceptual and the methodological bridge between micro-evolution and macro-evolution (e.g., Charlesworth, Lande & Slatkin, 1982; Coyne, 1992; Templeton, 1981; Futuyma, 1998; Wade, 2000). One aspect of reproductive isolation, hybrid dysfunction, often manifests as a breakdown of developmental systems

in hybrids (see below). For these reasons, we believe that the investigation of speciation is also a logical place to build a bridge between population genetic and evolution-of-development studies. Several questions basic to this synthesis come into focus here. What role do allelic changes in loci involved in regulatory pathways play in speciation and the further diversification of taxa? Can development, by facilitating reproductive isolation, play a creative role in the evolutionary process in addition to its role as constraining process (e.g., Wake, Roth & Wake, 1983)? which features of pathways (e.g., length of pathway, linear v.s. branched pathways) affect the likelihood that reproductive isolation will evolve?

Here, we will focus on the standard allopatric divergence model of speciation (Dobzhansky, 1937; Muller, 1942; Mayr, 1963), which is still widely accepted (e.g., Coyne, 1992; Johnson, 2000a; Lynch & Force, 2000). Other modes of speciation, such as 'ecological speciation' (e.g., Schulter, 1998, 2000; Hendry et al., 2000; Rundle & Whitlock, 2001) and sympatric speciation via host-race formation (e.g., Feder, 1998), will not be addressed. Current allopatric speciation models posit that interactions among alleles at different loci underlie hybrid fitness reduction (discussed in Orr, 1995; Gavrilets, 1997, 1999; Gavrilets, Li & Vose, 2000; Johnson, 2000a, b; Lynch & Force, 2000; Turelli & Orr, 2000 and references therein). This supposition follows from the classic models of Dobzhansky (1937) and Muller (1942) and is strongly supported by empirical studies (reviewed in Johnson, 2000a). These incompatibilities arise as the nascent species diverge but the nature of how they actually arise is not well understood.

In the neo-Darwinian framework, speciation, like evolution in general, is an opportunistic process (Dobzhansky, 1937; Mayr, 1963). That is, the divergence that leads to decreased hybrid fitness as a byproduct will probably occur in different manners and involve several different classes of genes and other heritable elements (Johnson, 1998). Moreover, the relative contributions of different classes of genes will probably vary in different taxa and under different circumstances. The opportunistic nature of speciation, however, does not argue against our thesis that divergence in gene regulation will play an important role in the process of speciation. Because gene regulation is a near-universal feature of development, regulatory interactions would likely play an important role in speciation. We present other reasons for our contention below.

The divergence resulting in hybrid incompatibility is itself adaptive or perhaps neutral. Only in the context of the changes in the genome of the other population, can this divergence decrease fitness. Johnson (2000a) contrasts the effects of alleles involved in hybrid incompatibility, calling their effect in a hybrid genetic background the negative phenotype and their effect in pure species, the positive phenotype. Provided the nascent species do not exchange genes, micro-evolutionary forces will act only upon the positive phenotype. The negative phenotypes, hybrid fitness reduction and other hybrid traits, will thus evolve solely as correlated characters (Johnson & Wade, 1996; Johnson, 2000a).

CJ Goodnight (unpubl. ms) showed that reproductive isolation can accrue between allopatric populations given epistatic genetic variance and directional selection. In Goodnight's model, selection converts nonadditive variance to additive variance and allows for the accumulation of genetic incompatibilities. Goodnight's model is general: with epistasis, directional selection, and allopatry, the evolution of reproductive isolation is likely. Gibson (1996) has shown via modeling the *Ubx* pathway that epistasis and pleiotropy are universal features of regulatory pathways and networks. In his analysis of Boolean (on/off) regulatory networks, Frank (1999) also finds epistatic interactions. Interactions in regulatory genetic pathways thus provide a biologically plausible mechanism for generating Dobzhansky–Muller genetic incompatibilities in hybrids.

Using individual-based simulations and analytical theory, we are examining speciation as a possible consequence of micro-evolutionary forces operating on phenotypes determined by interactions of genes in a linear, regulated pathway (Johnson & Porter, 2000; A.H. Porter & N.A. Johnson, manuscript in review). We presented our original model in the context of protein-DNA interactions (Johnson & Porter, 2000) but our framework is general, encompassing protein–protein and other regulatory interactions. In our pathway model, each locus has a regulatory and a product site (Figure 3). We assign numerical values for the allelic states of all products and promoters. For simple binding strength functions, the allelic states are each represented by a single number. We can also incorporate complexity in these functions by allowing each allelic state to be represented by two or more numbers. The phenotype is determined by the degree of match between the product site of each locus and the promoter site at the next. An individual's fitness

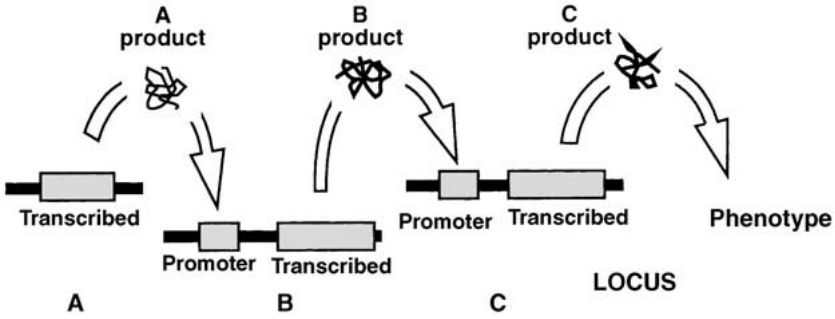

Figure 3. A simple, linear developmental genetic pathway, used to model the evolution of gene interactions. Binding of the protein from locus A to the promoter of locus B regulates how much of protein B is expressed. This in turn binds to the locus C promoter to regulate the amount of protein C. The amount of protein C is taken as the final phenotype. The phenotype evolves by modifying binding strengths along the pathway.

is determined by how well its phenotype matches the optimal phenotype. Individuals with phenotypes near the optimal phenotype have higher fitnesses than do those with more deviant phenotypes. We subject this overall phenotype to directional selection by having the optimal phenotype change with time. In our model, the response to selection occurs via multiple allelic changes at a few loci, similar to what is found in the fine-scale genetic studies of empirical model systems (Phillips, 1999; Stern, 2000; see §III above). Details can be found in Johnson and Porter (2000).

The salient results of our study include the following:

(1) Hybrid fitness reduction often evolves as a by-product of parallel adaptation if the trait under selection is produced by interactions between genes in a regulatory developmental genetic pathway.
(2) Hybrid fitness reduction does not evolve when the trait arises from additive (or multiplicative) gene action, despite directional selection acting on the trait.
(3) Hybrid fitness reduction does not evolve – at least on the time scales studied – in the absence of directional selection.
(4) In general, increasing either the number of genes in the pathway or the complexity of the binding site interactions increases the likelihood of obtaining reproductive isolation.
(5) In contrast, factors like population size and mutation rate only affect the results of our simulations slightly.

Recent studies of the *Odysseus* (*Ods*) locus support our contention that divergence of regulatory genetic pathways plays an important role in speciation. *Ods*, implicated in the sterility of hybrids between *D. simulans* and *D. mauritiana*, contains a homeodomain (Perez et al., 1993; Perez & Wu, 1995; Ting et al., 1998; Ting, Tsaur & Wu, 2000) at which extremely uneven rates of evolution have been observed (Ting et al., 1998; Ting, Tsaur & Wu, 2000). Specifically, there have been roughly the same number of amino acid substitutions at this site between the two sibling species *D. mauritiana* and *D. simulans* (separated by less than a million years; Hey & Kliman, 1993; Kliman et al., 2000) as between flies and mammals. This finding, combined with the high ratio of nonsynonymous to synonymous substitutions observed in the region, is strong evidence that directional selection has occurred at this homeodomain (Ting et al., 1998). Despite the multiple fixed differences between species at this locus, there is little within-species polymorphism (Ting, Tsaur & Wu, 2000). As Kliman et al. (2000, p. 1928) note, "this pattern is strongly suggestive of multiple recurrent selective sweeps at this locus". Recurrent selective sweeps is consistent with both our model of evolution in regulatory genetic pathways and the results from the very detailed, fine-scale genetic architecture studies (see above). *Ods*, of course, does not cause hybrid sterility *alone* but only in the genetic background of *D. simulans*. Genetic variation within *D. simulans* for the interactors of *Ods* has been identified and coarsely mapped (Perez, 1994). Furthermore, *Ods* from *D. mauritiana* in *D. simulans* genetic background does not cause complete sterility all by itself but requires the co-introgression of nearby 'helper factors' (Perez & Wu, 1995). The identities of the helper factors and interactors of *Ods* are not yet known but it would be interesting if they were all in the same regulatory genetic pathway. Nothing is yet known about the rates of evolution at these helper and interactor loci.

Other regulatory loci have been implicated in hybrid incompatibility in *Drosophila*. For instance, the

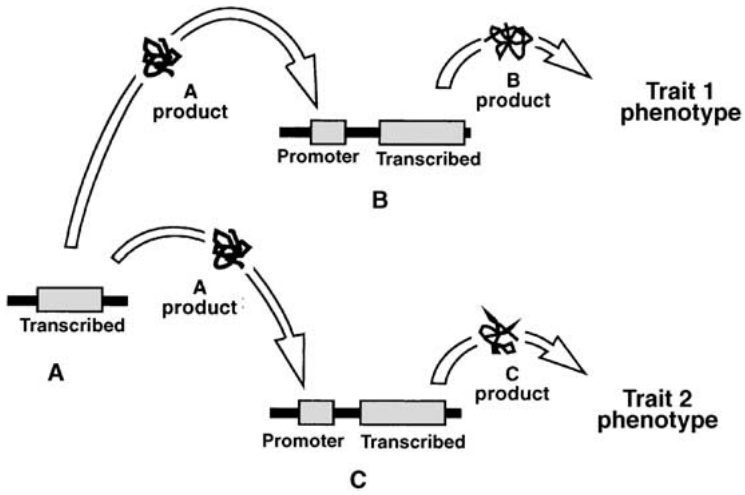

Figure 4. A simple, branched developmental genetic pathway. Protein from locus A binds to promoters of both locus B and locus C, and the strengths of binding regulate the amounts of the respective gene products. The amount of proteins B is taken as the phenotype of trait 1, and the amount of protein C is the phenotype of trait 2. Allelic variation at A affects variances and the covariance between traits 1 and 2, whereas allelic variation in loci B and C affect the variances but have little effect on the covariance.

absence of certain bristles in hybrids between *D. melanogaster* and *D. simulans* is due to mis-regulation of *Acheate-Scute* (Skaer & Simpson, 2000). Our simple two-locus model is essentially a mathematical representation of the schematic in Figure 2 of Skaer and Simpson (2000). In addition, Cyclin E, involved in cell-cycle regulation, plays a role in inviability of hybrids between *D. melanogaster* and *D. simulans* (Sawamura, Davis & Wu, 2000). Orr et al. (1997) also present evidence for cell-cycle defects causing inviability of interspecific hybrids in *Drosophila*.

Different species or populations sometimes possess the same phenotype but have different genotypes (Nanney, 1982; Cohan, 1984; Losos et al., 1998; see also Ludwig et al., 2000). In our simulations, allopatric populations under parallel, directional selection evolve toward the same phenotype but often via different underlying genetic changes (Johnson & Porter, 2000). This same result could be achieved, in theory, via mutation, drift, stabilizing selection, and compensatory mutations (Nei et al., 1983; Ludwig et al., 2000). Although we did not observe speciation when the trait was under just stabilizing selection, our simulations just covered a relatively short period of time.

Regulatory genetic pathways are typically more complex than the linear pathways we have thus far investigated. Often, pathways are branched, allowing one gene product to interact with the regulatory sites of more than one other gene. Do the properties that make linear, regulated genetic pathways conducive for the evolution of reproductive isolation also apply to the more complicated branched pathways? We predict that they do and present specific hypotheses and our reasoning below.

Hypothesis 1

In cases where three-locus, regulatory pathways are branched, leading to two different traits, with one trait under directional selection and the other held under stabilizing selection, reproductive isolation will occur at a frequency equal to that of the two-locus, linear pathway.

Rationale

Consider a three-locus, branched pathway (Figure 4) where the product of one locus (*A*) binds with regulatory elements of two loci (*B* and *C*). The interactions between loci *A* and *B* (interaction I) and between *A* and *C* (interaction II) result in separate traits, which can be under separate selection pressures. First consider the case where the trait 1 is under directional selection but the trait 2 is under stabilizing selection. The stabilizing selection on trait 2 would constrain locus *A* and thus nearly all of the response to selection on trait 1 would occur via changes in *B*'s regulatory elements. However, because binding depends upon the difference in the allelic values of the product and promoter sites, this scenario reduces to the two-locus (*A*–*B*) case. No matter how little locus *A* changed, provided sufficient

mutations occurred at the other locus, the populations would have opportunity to respond in different directions just as in the two-locus model (Johnson & Porter, 2000). We predict little change in a **G** matrix of these two traits. This same result should hold if locus *A* interacted with an arbitrary number of additional loci, each leading to traits under stabilizing selection.

Hypothesis 2

In branched pathways when more than one trait is under directional selection, reproductive isolation will occur at least as often (and perhaps more often) than in the two-locus, linear pathway case. The exact frequency will depend upon how many traits are under directional selection, the relative intensities of selection operating on each trait, and the exact manner in which the populations respond to selection.

Rationale

Consider the same three-locus, branched pathway (Figure 4), but now both traits are under directional selection. Responses must occur at both interaction I and interaction II. The question is the extent to which the changes at interaction I are independent to those at interaction II. The responses to selection for one trait may cause the allelic value of *A*'s product site to go up or down. A change in locus *A* then makes it more likely that the regulatory sites of locus *B* and locus *C* will evolve in the same direction, thus limiting the extent to which the populations can evolve in different and incompatible ways. At one extreme, where the changes at the different interactions are completely dependent, the branched pathway with two interactions under directional selection, then, should reduce to the two-locus linear pathway. At the other extreme, if each interaction is independent and the binding function is one dimensional, the expected frequency of hybrids with low fitness is (1/2 times 1/2) or 1/4. If the selection on one phenotype is stronger than on the other, we predict that more of the response will be along the interaction leading to the more strongly selected phenotype, and the degree of dependence of the two interactions will increase. We predict that the **G** matrix would evolve according to how selection operating on the two traits was correlated and thus would depend on the nature of ecological change. Under any of these scenarios, the logic presented above suggests that branched-pathway models can be reduced to variants of the linear pathway models we have been investigating.

VI. Toward the new synthesis

A centerpiece of this synthesis of population genetics and development will be a mechanistic theory of adaptation, one rooted in what we know about how phenotypes arise from genotypes. Such a theory would simultaneously consider quantitative effects of allelic change and population processes. While we have theories of adaptation (see Orr, 1998) and theories of the evolution in regulatory pathways (see Frank, 1999), we do not yet have a mechanistic theory that considers the quantitative, allelic variation involved in the processes that produce phenotypes from genotypes. Models involving binding-site interactions and allelic variation will form a basis for this synthesis. In addition to addressing evolutionary questions, such a theory would also address some of the fundamental questions of developmental biology, such as: Why is so much of development conserved? Such a theory would also advance the concept of developmental constraint in evolutionary biology, making it mechanistic rather than strictly phenomenological.

On the empirical side, we believe a central goal of such a synthesis should be determining the extent of allelic variation existing in the parts of molecules that have regulatory functions. These regulatory molecules include both DNA that can be bound and proteins that bind to DNA, RNA, and other proteins. The quantitative effects of this allelic variation on fitness and other traits also need to be accessed. Defining these effects even in simple laboratory systems will be useful in assisting the formation of the theory of adaptation and in providing insight for studies of more complex systems.

We envision collaborations between theoreticians and empiricists in examining the evolution of molecular co-option. In particular, we see the potential for progress in addressing the question: How many mutational steps are required for existing genetic variation in regulatory pathways to capturing new binding sites? Knowledge of the distribution of mutation effect sizes (see §III) for different types of regulatory genes will be important component in formulating an evolutionary theory of molecular co-option.

We also envision possible benefits to medicine emerging from this synthesis. For the most part, medicine has treated patients as populations and not individuals. Investigations of the allelic variation in developmental and physiological signaling pathways will aid in the design of drugs that take into account differences among individuals. The synthesis of

population genetics and development relies upon an understanding of the scope and effects of this natural variation.

Acknowledgements

We thank Andrew Hendry and Michael Kinnison for inviting us to participate in this forum. Günter Wagner, Patrick Phillips, and Michael Whitlock gave us permission to discuss their then-unpublished papers. We thank Scott Gilbert, Charles Goodnight, Mohamed Noor, Jeffrey Townsend, Michael Wade, Günter Wagner, the editors, and an anonymous reviewer for discussions and/or comments on the paper. While we did not always follow their advice, their suggestions were consistently insightful. We are grateful for the support from the National Science Foundation (DEB 0075451).

References

Arnold, S.J., 1983. Morphology, performance, and fitness. Am. Zool. 23: 347–361.

Arnold, S.J., M.E. Pfrender & A.G. Jones, 2001. The adaptive landscape as a conceptual bridge between microevolution and macroevolution. Genetica 112–113: 9–32.

Arnold, S.J. & P.C. Phillips, 1999. Hierarchical comparison of genetic variance–covariance matrices. II. Coastal-inland divergence in the garter snake, *Thamnophis elegans*. Evolution 53: 1516–1527.

Arnone, M.I. & E.H. Davidson, 1997. The hardwiring of development: organization and function of genomic regulatory elements. Development 124: 1851–1864.

Barton, N.H. & M. Turelli, 1989. Evolutionary quantitative genetics: How little do we know? Ann. Rev. Genet. 23: 337–370.

Bender, W., M. Akjam, F. Karch, P.A. Beachy, M. Peifer, P. Spierer, E.B. Lewis & D.S. Hogness, 1983. Molecular genetics of the *bithorax* complex in *D. melanogaster*. Science 221: 23–29.

Boguski, M.S., 1999. Biosequence exegesis. Science 286: 453–455.

Bradshaw, H.D., Jr., K.G. Otto, B.E. Frewen, J.K. McKay & D.W. Schemske, 1998. Quantitative trait loci affecting differences in floral morphology between two species of monkeyflower (*Mimulus*). Genetics 149: 367–382.

Bryant, E.H., S.A. McCommas & L.M. Coombs, 1986. The effect of an experimental bottleneck upon quantitative genetic variation in the housefly. Genetics 114: 1191–1223.

Charkesworth, B., R. Lande & M. Slatkin, 1982. A neo-Darwinian commentary on macroevolution. Evolution 36: 474–498.

Cohen, F.M., 1984. Genetic divergence under uniform selection. I. Similarity among populations of *Drosophila melanogaster* in their responses to artificial selection for modifiers of ci^D. Evolution 38: 55–71.

Conway Morris, S., 2000. Evolution: bringing molecules into the fold. Cell 100: 1–11.

Coyne, J.A., 1992. Genetics and speciation. Nature 355: 511–515.

Coyne, J.A., 1995. Speciation in monkeyflowers. Nature 376: 726–727.

Darwin, C., 1859. On the Origin of Species. John Murray, London.

Davidson, E.H., 1986. Gene Activity in Early Development. Academic Press, San Diego, 3rd edn.

Dobzhansky, Th., 1937. Genetics and the Origin of Species. Columbia University Press, New York.

Ephrussi, B., 1958. The Cytoplasm and Somatic Cell Variation. J. Cell. Compar. Physiol. 52 (suppl.): 35–54.

Falconer, D.S. & T.F.C. MacKay, 1996. Introduction to Quantitative Genetics. Longman, Harlow, U. K., 4th edn.

Feder, J.L., 1998. The Apple Maggot fly, *Rhagoletis pomonella*: flies in the face of conventional wisdom about speciation?, pp. 130–144 in Endless Forms: Species and Speciation, edited by D.J. Howard & S.H. Berlocher. Oxford University Press, New York.

Feder, M.E. & W.B. Watt, 1992. Functional biology of adaptation, pp. 365–392 in Genes in Ecology, edited by R. J. Berry, T. J. Crawford & G.M. Hewitt. Blackwell Scientific Publications, Oxford.

Felsenstein, J., 1985. Phylogenies and the comparative method. Amer. Natur. 125: 1–15.

Fields, S., 2001. Proteomics – proteomics in genomeland. Science 291: 1221.

Fisher, R.A., 1930. The Geneticial Theory of Natural Selection. Dover Press, New York.

Frank, S.A., 1999. Population and quantitative genetics of regulatory networks. J. Theor. Biol. 197: 281–294.

Futuyma, D. J., 1998. Evolutionary Biology. Sinauer Associates, Sunderland, M.A., 3rd edn.

Garstang, W., 1922. The theory of recapitulation: a critical restatement of the biogenetic law. J. Linn. Soc. Zool. 35: 81–101.

Gavrilets, S., 1997. Evolution and speciation on holey adaptive landscapes. Trends Ecol. Evol. 12: 307–312.

Gavrilets, S., 1999. A dynamical theory of speciation on holey adaptive landscapes. Amer. Natur. 154: 1–22.

Gavrilets, S., H. Li & M.D. Vose, 2000. Patterns of parapatric speciation. Evolution 54: 1126–1134.

Gerhart, J. & M. Kirschner, 1997. Cells, Embryos, and Evolution. Blackwell Science.

Gibson, G., 1996. Epistasis and pleiotropy as natural properties of transcriptional regulation. Theor. Pop. Biol. 49: 58–89.

Gilbert, S.F., 1988. pp. 311–346. in The American Development of Biology, edited by R. Rainger, K.R. Benson & J. Maienschein. University of Pennsylvania Press, Philadelphia, P.A.

Gilbert, S.F., 1997. Developmental Biology. Sinauer Press, Sunderland, M.A., 5th edn.

Gilbert, S.F., 2000. Genes classical and genes developmental: the different uses of the gene in evolutionary syntheses, pp. 178–192 in The Concept of the Gene in Development and Evolution, edited by P. Buerton, R. Falk & H.-J. Rheinberger. Cambridge University Press, Cambridge, U.K.

Gilbert, S.F., J.M. Opitz & R.A. Raff, 1996. Resynthesizing evolutionary and developmental biology. Devel. Biol. 173: 357–372.

Goldschmidt, R., 1940. The Material Basis of Evolution. Yale University Press, New Haven, C.T.

Goodnight, C.J., 1987. On the effect of founder events on epistatic genetic variance. Evolution 41: 80–91.

Gromko, M.H., 1995. Unpredictability of correlated response to selection – pleiotropy and sampling interact. Evolution 49: 685–693.

Halder, G., P. Callaerts & W.J. Gehring, 1995. Induction of ectopic eyes by targeted expression of the *eyeless* gene in *Drosophila*. Science 267: 1788–1792.

Hansen, T.F. & G.P. Wagner, 2001. Modeling genetic architecture: a multilinear theory of gene interaction. Theor. Pop. Bio. 59: 61–86.

Hendry, A., J.K. Wenburg, P. Bentzen, E.C. Volk & T.P. Quinn, 2000. Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. Science 290: 516–518.

Hey, J. & R.M. Kliman, 1993. Population genetics and phylogenetics of DNA sequence variation at multiple loci within the *Drosophila melanogaster* species complex. Mol. Biol. Evol. 10: 804–822.

Hillis, D.M., C. Moritz & B. Mable, 1996. Molecular Systematics. Sinauer Associates, Sunderland, M.A., 2nd edn.

Johnson, N.A., 1998. Postzygotic reproductive isolation: epigenetics for an epiphenomenon? J. Evol. Biol. 11: 207–212.

Johnson, N.A., 2000a. Gene interaction and the origin of species, pp. 197–212 in Epistasis and the Evolutionary Process, edited by J.B. Wolf, E.D. Brodie III & M.J. Wade. Oxford University Press, New York.

Johnson, N.A., 2000b. Speciation: Dobzhansky–Muller incompatibilities, dominance, and gene interaction. Trends Ecol. Evol. 15: 480–482.

Johnson, N.A. & A.H. Porter, 2000. Rapid speciation via parallel, directional selection on regulatory genetic pathways. J. Theor. Biol. 205: 527–542.

Johnson, N.A. & M.J. Wade, 1996. Genetic covariances within and between species: indirect selection for hybrid inviability. J. Evol. Biol. 9: 205–214.

Jones, S., 1999. Darwin's Ghost: 'The Origin of Species' Updated. Ballantine, New York.

Kauffman, S., 1993. The Origins of Order: Self-Organization and selection in Evolution. Oxford University Press, New York.

Kliman, R.M., P. Andolfatto, J.A. Coyne, F. Depaulis, M. Kreitman, A.J. Berry, J. McCarter, J. Wakeley & J. Hey, 2000. The population genetics of the origin and divergence of the *Drosophila simulans* complex species. Genetics 156: 1913–1931.

Kohler, R.E., 1994. Lords of the Fly: *Drosophila* Genetics and Experimental Life. University of Chicago Press, Chicago.

Lascoux, M., 1997. Unpredictability of correlated response to selection: linkage and initial frequency also matter. Evolution 51: 1394–1400.

Lewin, B., 1997. Genes VI. Oxford University Press, Oxford.

Lewis, E.B., 1978. A gene complex controlling segmentation in *Drosophila*. Nature 276: 565–570.

Lewontin, R.C., 1974. The Genetic Basis of Evolutionary Change. Harvard University Press.

Losos, J.B., T.R. Jackman, A. Larson, K. de Queiroz & L. Rodriguez-Schettino, 1998. Contingency and determinism in replicated adaptive radiations of island lizards. Science 279: 2115–2118.

Ludwig, M.Z., C. Bergman, N.H. Patel & M. Kreitman, 2000. Evidence for stabilizing selection in a eukaryotic enhancer element. Nature 403: 564–567.

Lyman, R.F. & T.F.C. MacKay, 1998. Candidate quantitative trait loci and naturally occurring phenotypic variation for bristle number in *Drosophila melanogaster*: the *Delta-Hairless* gene region.

Lynch, M. & A.G. Force, 2000. The origin of interspecific genomic incompatibility via gene duplication. Amer. Natur. 156: 590–605.

Lynch, M. & J.B. Walsh, 1998. Fundamentals of Quantitative Genetics. Sinauer Associates, Sunderland, M.A.

Mackay, T.F.C., 1995. The genetic basis of quantitative variation: numbers of sensory bristles of *Drosophila melanogaster* as a model system. Trends Genet. 11: 464–470.

Mackay, T.F.C., 1996. The nature of quantitative genetic variation revisited: lessons from *Drosophila* bristles. BioEssays 18: 113–121.

MacKay, T.F.C. & C.H. Langley, 1990. Molecular and phenotypic variation in the *achaete-scute* region of *Drosophila melanogaster*. Nature 348: 64–66.

Martins, E.P. (ed.), 1996. Phylogenies and the Comparative Method in Animal Behavior. Oxford University Press, New York.

Mayr, E., 1963. Animal Species and Evolution. Harvard University Press, Cambridge, M.A.

Mayr, E., 1982. The Growth of Biological Thought. Harvard University Press, Cambridge, M.A.

McGinnis, W.C., R.L. Garber, J. Wirz, A. Kurioiwa & W.J. Gehring, 1984a. A homologous protein-coding sequence in *Drosophila* homeotic genes and its conservation in other metazoans. Cell 37: 403–408.

McGinnis, W., M.S. Levine, E. Hafen, A. Kuroiwa & W. Gehring, 1984b. A conserved DNA sequence in homeotic genes of the *Drosophila antennapedia* and *Bithorax* complexes. Nature 308: 428–433.

McPeek, M., 1995. Testing hypotheses about evolutionary change on single branches of a phylogeny using evolutionary contrasts. Am. Nat. 145: 686–703.

Muller, H.J., 1942. Isolating mechanisms, speciation, and temperature. Biol. Symp. 6: 71–125.

Munn, J.S. & R. Kopan, 2000. Notch signaling: from the outside in. Devel. Biol. 228: 151–165.

Nagy, L., 1998. Changing patterns of gene regulation in the evolution of arthropod morphology. Amer. Zool. 38: 818–828.

Nanney, D.L., 1982. Genes and phenes in *Tetrahymena*. Bioscience 783–788.

Nusselin-Volhard, C. & E.Wieschaus, 1980. Mutations affecting segmentation number and polarity in *Drosophila*. Nature 287: 795–801.

Orr, H.A., 1995. The population genetics of speciation: the evolution of hybrid incompatibilities. Genetics 139: 1805–1813.

Orr, H.A., 1998. The population genetics of adaptation: the distribution of factors fixed during adaptive evolution. Evolution 52: 935–949.

Orr, H.A., L.D. Madden, J.A. Coyne, R. Goodwin & R.S. Hawley, 1997. The developmental basis of hybrid inviability: a mitotic defect in *Drosophila* hybrids. Genetics 145: 1031–1040.

Orr, H.A. & J.A. Coyne, 1992. The genetics of adaptation: a reassessment. Amer. Natur. 140: 725–742.

Palopoli, M.F. & N.H. Patel, 1996. Neo-Darwinian developmental evolution: can we bride the gap between pattern and process? Curr. Opin. Genet. Devel. 6: 502–508.

Pederson, J.A., J.W. La Follette, C. Gross, A. Veraksa, W. McGinnis & J.W. Mahaffey, 2000. Regulation by homeoproteins: a comparsion of deformed-responsive elements. Genetics 156: 677–686.

Perez, D.E., 1994. Genetics of postmating reproductive isolation in *Drosophila*: investigation of an X-linked hybrid sterility region. PhD Dissertation, University of Chicago, Chicago, I.L.

Perez, D.E. & C.-I. Wu, 1995. Further characterization of the *Odysseus* locus of hybrid sterility in *Drosophila*: one gene is not enough. Genetics 140: 201–206.

Perez, D.E., C.-I. Wu, N.A. Johnson & M.-L. Wu, 1993. Genetics of reproductive isolation in the *Drosophila simulans* clade: DNA marker-assisted mapping of a hybrid-male sterility gene, *Odysseus* (*Ods*). Genetics 134: 261–275.

Phillips, P.C., 1999. From complex traits to complex alleles. Trends Genet. 15: 6–8.

Phillips, P.C. & S.J. Arnold, 1999. Hierarchical comparison of genetic variance–covariance matrices. I. Using the Flury hierarchy. Evolution 53: 1506–1515.

Phillips, P.C., M.C. Whitlock & K. Fowler, 2001. Inbreeding changes the shape of the genetic covariance matrix in *Drosophila melanogaster*. Genetics (in review).

Pigliucci, M. & C.D. Schlichting, 1997. On the limits of quantitative genetics for the study of phenotypic evolution. Acta Biotheor. 45: 143–160.

Pray, L.A. & C.J. Goodnight, 1995. Genetic variability in inbreeding depression in the flour beetle, *T. castaneum*. Evolution 49: 176–188.

Quiring, R., U. Walldorf, U. Kloter & W.J. Gehring, 1994. Homology of the *eyeless* gene of *Drosophila* to the *Small eye* gene in mice and *Anirida* in humans. Science 265: 785–789.

Raff, R.C., 1996. The Shape of Life. University of Chicago Press, Chicago.

Rice, S.H., 1998. The evolution of canalization and the breaking of von Baer's laws: modeling the evolution of development with epistasis. Evolution 52: 647–656.

Rice, S.H., 2000. The evloution of developmental interactions, pp. 82–98 in Epistasis and the Evolutionary Process, edited by J.B. Wolf, E.D. Brodie III & M.J. Wade. Oxford University Press, New York.

Roff, D.A., 2000. The evolution of the **G** matrix: selection or drift? Heredity 84: 135–142.

Roff, D.A. & T.A. Mousseau, 1999. Does natural selection alter genetic architecture? An evaluation of quantitative genetic variation among populations *of Allonemobius socius* and *A. fasciatus*. J. Evol. Biol. 12: 361–369.

Rundle, H.D. & M.C. Whitlock, 2001. A genetic interpretation of ecologically dependent isolation. Evolution 55: 198–201.

Sawamura, K., A.W. Davis & C.-I. Wu, 2000. Genetic analysis of speciation by means of introgression into *Drosophila melanogaster*. Proc. Nat. Acad. Sci. U.S.A. 97: 2652–2655.

Schemske, D.W. & H.D. Bradshaw, Jr., 1999. Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). Proc. Natl. Acad. Sci. U.S.A. 96: 11910–11915.

Schulter, D., 1998. Ecological causes of speciation, pp. 114–129 in Endless Forms: Species and Speciation, edited by D.J. Howard and S.H. Berlocher. Oxford University Press, New York.

Schulter, D., 2000. The Ecology of Adaptive Radiation. Oxford University Press, New York.

Schutt, C. & R. Nothiger, 2000. Structure, function, and evolution of sex-determining systems in Dipeteran insects. Development 127: 667–677.

Scott, M.P. & A.J. Weiner, 1984. Structural relationships among genes that control development: sequence homology between the *Antennapedia, Ultrabithroax*, and *fushi tarazu* loci of *Drosophila*. Proc. Natl. Acad. Sci. U.S.A. 81: 4115–4119.

Skaer, N. & P. Simpson, 2000. Genetic analysis of bristle loss in hybrids between *Drosophila melanogaster* and *D. simulans* provides evidence for divergence of *cis*-regulatory sequences in the *achaete-scute* gene complex. Devel. Biol. 221: 148–167.

Stern, D., 2000. Perspective: evolutionary developmental biology and the problem of variation. Evolution 54: 1079–1091.

Sucena, E. & D.L. Stern, 2000. Divergence of larval morphology between *Drosophila sechellia* and its sibling species caused by *cis*-regulatory evolution of *ovo/shaven-baby*. Proc. Natl. Acad. Sci. U.S.A. 97: 4530–4534.

Templeton, A.R., 1981. Mechanisms of speciation – a population genetic approach. Ann. Rev. Ecol. Syst. 12: 23–48.

Thompson, D.W., 1942. On Growth and Form. Cambridge University Press, Cambridge, U.K.

Ting, C.-T., S.-C. Tsaur, M.-L. Wu & C.-I. Wu, 1998. A rapidly evolving homeobox at the site of a hybrid sterility gene. Science 282: 1501–1504.

Ting, C.T., S.C. Tsaur & C.-I. Wu, 2000. The phylogeny of closely related species as revealed by the genealogy of a speciation gene, *Odysseus*. Proc. Natl. Acad. Sci. U.S.A. 97: 5313–5316.

Turelli, M. & H.A. Orr, 2000. Dominance, epistasis, and the genetics of postzygotic isolation. Genetics 154, 1663–1679.

Wade, M.J., 2000. pp. 213–231 in Epistasis and the Evolutionary Process, edited by J.B. Wolf, E.D. Brodie III & M.J. Wade. Oxford University Press, New York.

Wagner, G.P., 1996. Does evolutionary plasticity evolve? Evolution 50: 1008–1023.

Wake, D.B., G. Roth & M. Wake, 1983. On the problem of stasis in organismal evolution. J. Theor. Biol. 101: 211–224.

Wolf, J.B., W.A. Frankino, A.F. Agrawal, E.D. Brodie III & A.J. Moore, 2001. Developmental interactions and the constituents of quantitative variation. Evolution 55: 232–245.

Wright, S., 1968. Evolution and the Genetics of Populations. Vol. I., Genetic and Biometric Foundations. University of Chicago Press, Chicago.