Genetic Polymorphism in Heterogeneous Environments: The Age of Genomics

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Abstract

The selective mechanisms for maintaining polymorphism in natural populations has been the subject of theory, experiments, and review over the past half century. Advances in molecular genetic techniques have provided new insight into many examples of balancing selection. In addition, new theoretical developments demonstrate how diversifying selection over environments may maintain polymorphism. Tests for balancing selection in the current generation, the recent past, and the distant past provide a comprehensive approach for evaluating selective impacts. In particular, sequenced-based tests provide new ways to evaluate the long-term impact of selection on particular genes and the overall genome in natural populations. Overall, there appear to be many loci exhibiting the signal of adaptive directional selection from genomic scans, but the present evidence suggests that the proportion of loci where polymorphism is maintained by environmental heterogeneity is low. However, as more molecular genetic details become available, more examples of polymorphism maintained by selection in heterogeneous environments may be found.

INTRODUCTION

In 1976 and 1986, I wrote reviews (Hedrick 1986, Hedrick et al. 1976; see also Felsenstein 1976) discussing the maintenance of genetic polymorphism in heterogeneous (variable) environments. At that time, most of the well-known examples of genetic polymorphism that appeared to be maintained by diversifying selection were either visible or allozyme polymorphisms. Here, I revisit some of these examples, particularly ones for which there have been recent developments, such as relevant DNA sequence information, and I discuss some new examples. In my previous reviews, theory suggested that maintenance of polymorphism was possible in variable environments but that it was most likely when selection varied in space and/or there was limited gene flow or habitat selection. Here, I discuss some new theoretical developments related to these ideas and some other theory related to selection in variable environments.

Overall, the topics in this review are somewhat changed from my previous reviews. In my earlier reviews, an important issue was the evaluation of experimental studies, mainly in *Drosophila*, examining genetic perturbations, environmental perturbations, and habitat selection, but in recent years, the emphasis on these studies has declined. A new focus has been the emphasis on using DNA sequence data to identify and understand the impact of selection, particularly favorable directional selection, on specific genes. If there is favorable selection for different alleles in different environments, then diversifying selection may occur. I discuss some of this research and how it can be used to identify genes under diversifying selection.

GENERAL EXAMPLES

How important is variable selection in space and/or time in maintaining genetic polymorphism at specific genes? Below, I discuss some of best examples, but the overall number for which there is detailed information is limited. There are a substantial number of examples for which directional selection has been or is important (note that several other examples are discussed as illustrations in the section on theory). Within a given species, present evidence suggests that polymorphism is maintained by diversifying selection at only a few genes. And, except for some genes related to pathogen resistance, these appear to be maintained only in the given species. If polymorphisms are in related species, researchers have often found a separate origin for the polymorphism. In other words, many of these polymorphisms are relatively recent and not maintained over the formation of new species.

I begin with comments about recent investigations in some well-known, or particularly relevant, examples of genetic polymorphism in heterogeneous environments. Because of space limitations, I do not discuss shell polymorphism in the land snail *Cepaea nemoralis* (Cook 1998, Jones et al. 1977) or the extensive experimental work in microorganisms (except for one example in *Pseudomonas fluorescens* below), but as entry into this literature, I recommend the following: Suiter et al. (2003), Elena & Lenski (2003), Zhong et al. (2004), Dykhuizen & Dean (2004), Brisson & Dykhuizen (2004), MacLean (2005), Barrett et al. (2005).

Color Polymorphism

Melanism in *Biston betularia*. A classic example of a genetic polymorphism influenced by diversifying selection is industrial melanism in the peppered moth, *Biston betularia* (Cook 2003, Grant 2005, Kettlewell 1973, Majerus 1998). Industrial melanics were first noticed in England in the mid-nineteenth century and appear to have rapidly spread from a single source. At a particular location, the increase from a low frequency to a frequency greater than 90% often took only several decades. The main selective agent appears to have been differential predation by birds on polluted and unpolluted resting backgrounds, and extensive gene flow significantly influenced the spread and distribution of melanics (Cook 2003). The overall case for natural selection acting on the rise and fall of melanics in peppered moths is indisputable.

Since the introduction of clean air legislation in the 1960s in Great Britain, the frequency of dominant melanic moths has declined. For example, at Caldy Common, the frequency of melanics declined from greater than 90% in 1960 to below 5% by 2002 (**Figure 1**) (Grant 2005). Also given in **Figure 1** is the very similar expected decrease of the melanic phenotype, assuming a 15.3% selective disadvantage for melanics [that is, the fitnesses for the dominant melanics and recessive typicals are 0.847 and 1.0, respectively (Grant et al. 1996)].

B. betularia is also found in North America, and in some areas the frequency of melanics once was quite high (Grant et al. 1996). In the 1960s, clean air legislation also began to result in better air quality in North America, and the frequency of melanics dropped (Grant & Wiseman 2002). Presumably, similar reversals in selection pressures that occurred on both continents were responsible for this remarkable parallel decline. On the basis of crosses between American and British moths, researchers concluded that the melanic variants from the two continents are alleles at the same locus (Grant 2004). When the gene that determines melanism in *B. betularia* is discovered, then DNA data should be able to determine the cause and relationship

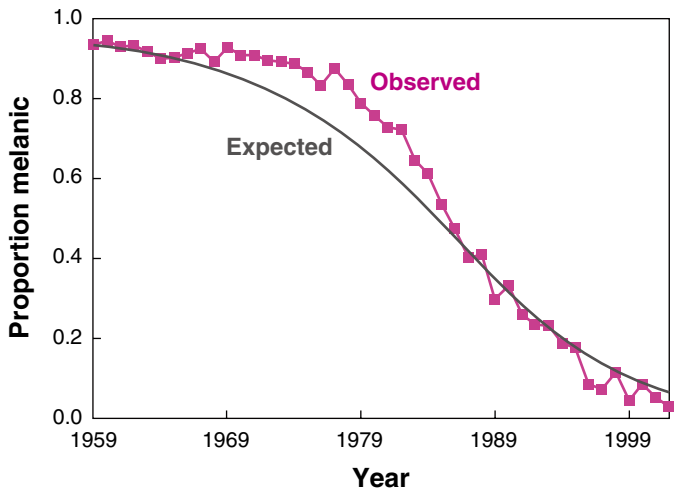

Figure 1

The observed decline in the frequency of melanics from 1959 to 2002 at Caldy Common in England (*magenta squares*) (Grant 2005) and the expected decline when there is 15.3% selection against the melanics (*dark gray line*).

of melanism in different populations, the number of different melanic mutants, their age, what amino acid changes cause melanism, and related factors.

Melanism in mice. From studies of coat color mutants in house mice, researchers identified a number of genes that can cause melanism. However, several molecularly documented cases of melanism in wild vertebrate populations appear to be the result of variation in the melancortin-1 receptor gene (*Mc1r*), including in birds (Mundy 2005), in mammals (Eizirik et al. 2003, Nachman et al. 2003), and in reptiles (Rosenblum et al. 2004).

Nachman et al. (2003) presented a detailed examination of melanism that is due to *Mc1r* variation in the rock pocket mouse (*Chaetodipus intermedius*), a light-colored mouse that generally lives on light-colored granite rocks but has melanic forms that live on black lava in several restricted sites in the southwestern United States. **Figure 2** shows the frequencies of the normal recessive and dominant melanic forms from a 35-km transect in southwestern Arizona (Hoekstra et al. 2004). Here, the frequency of melanics is highly concordant with substrate color: High frequencies of melanics occurred in the center of this transect, which has approximately 10 km of black

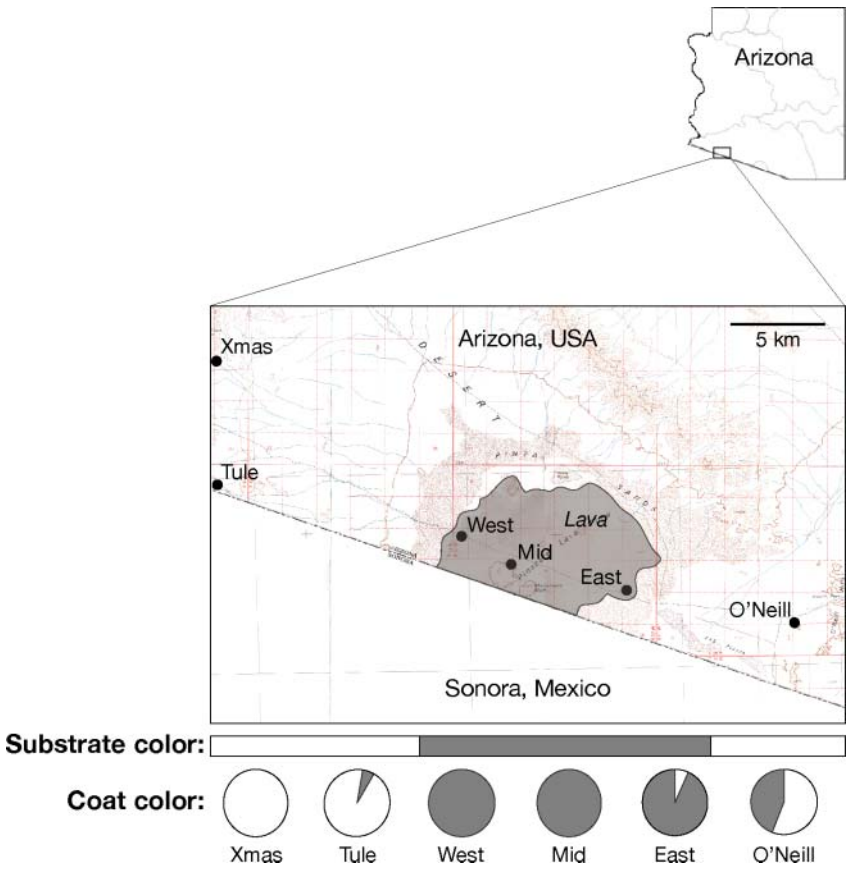

Figure 2

Six sampling sites (three on dark volcanic rock and three on light-colored substrate) and coat color frequencies (in pie diagrams) in rock pocket mice across a transect in the Sonoran desert (Hoekstra et al. 2004).

lava, and lower frequencies of melanics occurred on the light-colored substrate sites at either end of the transect. Overall, 95% of the mice were melanic on the three lava sites, and 23% of the mice were melanic in the three sites with light-colored substrate (most of these melanics were from the O'Neill sample, which, based on mtDNA sequence data, had the most gene exchange with the lava sites). There was no association of mtDNA variation and background color in these samples, and estimates of gene flow for neutral mtDNA markers were substantial.

Investigation of molecular variation in the *Mc1r* gene, which is known to have variants that produce dark-colored house mice, was found to correlate nearly completely with the light and melanic phenotypes. The melanic and normal alleles were found to differ by four amino acid differences (Nachman et al. 2003), and the nucleotide diversity for the melanic alleles was 1/20th that for light alleles. The lower variation among the melanic alleles is consistent with the expected pattern if selection has recently increased its frequency. Although variants at *Mc1r* appear to determine melanism patterns in this instance, in another population with melanics in New Mexico, *Mc1r* was not the gene responsible.

Recently, Hoekstra et al. (2006) identified a single nucleotide mutation in *Mc1r* that is a major determinant of the derived light color in a recent (<6,000 years old) "beach mouse" subspecies of *Peromyscus polionotus*. Matching of background color with pelage coloration for concealment appears to be the selective mechanism. However, in other beach mice, the light color does not appear to be determined by *Mc1r*. A single amino acid change in another gene (*SLC24A5*) has been shown to have a major influence on the difference in skin color between African and European human populations (Lamason et al. 2005). In this case, there is strong evidence for the recent evolution of the light color variant, such as very low heterozygosity for linked genes on haplotypes of the light color variant. These studies of the genetics of color differences within species are providing some of the most convincing examples of strong adaptive selection in different environments.

Pesticide Resistance

Some of the best-documented examples of selection in heterogeneous environments are those resulting from recent human changes in the environment, such as the use of chemicals to control pests (McKenzie 1996). The genetic basis of insecticide resistance may be the result of many genes (particularly in laboratory-selected strains), of mutants at a single or a few genes (ffrench-Constant et al. 2004, Oakeshott et al. 2003), or of expansion of gene families (Labbe et al. 2005, Ranson et al. 2002). Because the molecular basis of many of these genes is known, detailed genetic and evolutionary understanding is possible.

Insects. Resistance to some insecticides among mosquitoes that are vectors for diseases such as malaria (*Anopheles gambiae*) and West Nile virus (*Culex pipiens*) are the result of single amino acid substitutions. For example, a single nucleotide change, GGC (glycine) to AGC (serine) at codon position 119 in the gene for the enzyme acetylcholin-esterase (*ace*-1), in *C. pipiens* results in insensitivity to organophosphates

(Weill et al. 2003). A complete lack of variation within samples among resistant haplotypes suggests that they have originated and spread quite recently. In addition, the mutated amino acid is in the active "gorge" of the enzyme, and the same catalytic properties and insecticidal sensitivity were present in transfected mosquitoes (Weill et al. 2003).

Instead of undergoing a qualitative change, some insects become resistant to insecticides by producing large amounts of detoxifying enzymes. For example, the overtranscription of a single allele at a single cytochrome P450 gene in *Drosophila melanogaster* has been shown to confer worldwide resistance to DDT (Daborn et al. 2002). In this case, microarray analysis of all the P450 genes identified the specific overtranscribed gene [although further analysis (Pedra et al. 2004) suggested that the resistance is more complicated], and subsequent genetic and sequence investigation demonstrated that the single resistance allele resulted from the insertion of an *Accord* transposable element in the 5′ end of the gene. A survey of 34 populations worldwide found a complete correspondence of *Accord* insertion and insecticide resistance (Catania et al. 2004). Another example of an insertion of a transposable element adaptive to organophosphate pesticides by truncation of a gene has recently been documented (Aminetzach et al. 2005).

Warfarin resistance in rats. Warfarin and related anticoagulants were introduced in the early 1950s to control rodent pests, such as the Norway or brown rat, *Rattus norvegicus*, a widespread agricultural and domestic pest. However, resistance to warfarin evolved quickly, and resistant rats (both brown and black) and house mice are now found throughout the United States and Europe (Pelz et al. 2005). Resistance in brown rats is the result of a dominant allele *R* at an autosomal locus *Rw*, and the resistant animals are generally heterozygotes for *R* and for the wild-type, susceptible (*S*) allele. Warfarin inhibits blood coagulation by repressing the vitamin K reductase reaction (VKOR). *Rw* has recently been identified as the gene *VKORC1* encoding for a protein in the VKOR pathway (Rost et al. 2004). Missense mutations in this protein have been found in warfarin-resistant humans and rats and have reduced enzyme activity.

In an area along the England-Wales border, *RR* homozygotes had low viability because of a 20-fold increase in vitamin K requirement (Greaves et al. 1977). When warfarin was applied, a net heterozygote advantage resulted, and the relative survivals of genotypes *RR*, *RS*, and *SS* were estimated as 0.37, 1.0, and 0.68, respectively. In one population, the frequency of *R* was stable and polymorphic over nearly a decade. When warfarin was no longer applied in another population, the frequency of *R* declined very quickly, a result that illustrated the cost of maintaining the resistance allele.

Kohn et al. (2000, 2003) attempted to locate the warfarin resistance gene (*Rw*) and characterize variation at closely linked markers in German rat populations, which have varying levels of warfarin resistance. They determined that there has been a single and recent origin of warfarin resistance in these populations. On the basis of indirect genetic evidence, researchers determined that heterozygote advantage is not present in the German populations perhaps because these resistant rats do not suffer vitamin K deficiency, as documented in other populations (Markussen et al. 2003).

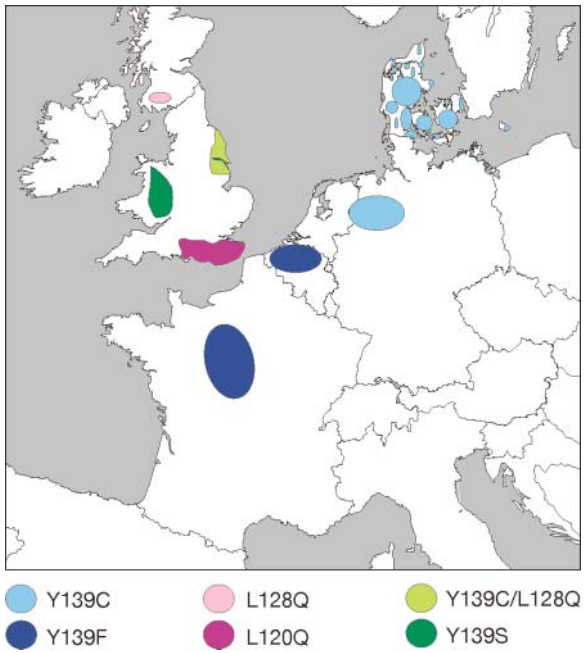

Figure 3

Geographic distribution of warfarin-resistant brown rats in Europe (Pelz et al. 2005). The areas where the six different resistant mutants have been found are indicated by different colors (indicated by ancestral amino acid, amino acid site, and derived amino acid).

In other words, the net heterozygote advantage for warfarin resistance appears to be present for some resistance alleles (populations) and not for others.

Recently, Pelz et al. (2005) have characterized *VKORC1* resistant variants in wild brown rat populations over much of Europe (**Figure 3**). They found eight different mutations, five of which affected only the two amino acids at positions 128 and 139. The mutant Y139F was found in both France and Belgium populations, and another mutant Y139C was found in both Germany and Denmark populations. The variant previously found to exhibit heterozygote advantage along the England-Wales border was a third variant, Y139S, at the same amino acid position. Because new amino acids are found at position 139 for so many resistance alleles, Pelz et al. speculated that Y139 may be part of the warfarin binding site of *VKORC1*. Because these variants have reached substantial frequency in only a few decades, they likely are not the result of new mutants, but rather were already present in these populations at low frequency.

Pathogen Resistance

Major histocompatibility complex (MHC). The MHC genes are part of the immune system in vertebrates, and differential selection through resistance to pathogens is widely thought to be the basis of their high genetic variation (Edwards & Hedrick 1998, Hedrick & Kim 2000, Hughes 1999, Meyer & Thomson 2001, Piertney & Oliver 2006). I provide a brief introduction here because I use MHC genes below as an example of balancing selection. Variation in the genes of the human MHC, known as *HLA* genes, have been the subject of intensive study for many years because of their role in acceptance or rejection of transplanted organs, in many autoimmune diseases,

and in recognition of pathogens. With sequence-determined alleles in worldwide human surveys, genes *HLA-A*, *HLA-B*, and *HLA-DR1* have 243, 499, and 321 alleles, respectively (Garrigan & Hedrick 2003), with *HLA-B* being the most variable gene in the human genome (Mungall et al. 2003).

In addition, most of the nucleotide variation is in functionally important parts of the MHC genes and results in amino acid variation. For example, Hedrick et al. (1991) determined the amino acid heterozygosity for the 366 (*HLA-A*) or 363 (*HLA-B*) amino acid sites in humans. There were a number of individual amino acid sites that have heterozygosities greater than 50% for both genes, and most of these amino acid sites appear, based on the three-dimensional structure of the HLA molecule, to have important functions involved with initiating the immune response. Similarly, high heterozygosity for functional amino acid sites has been found for other *HLA* genes (Salamon et al. 1999) and for MHC genes in the Arabian oryx (Hedrick et al. 2000) and wolves (Hedrick et al. 2002).

CCR5 and disease resistance. An apparent adaptive selection example is the 32-base pair coding sequence deletion of the human chemokine receptor 5, *CCR5-Δ32*. From population surveys, homozygotes for the deletion are resistant to HIV infection, and heterozygotes have a delayed onset of AIDS (Samson et al. 1996). This variant has a quite nonuniform geographical distribution, with a relative high frequency, particularly for a null allele, in northern Europe (16% in some areas) and a lower frequency in southern Europe (4% in Greece), and it has not been found in African and Asian samples (Novembre et al. 2005).

Using theoretical approaches and the observed amount of linkage disequilibrium at two nearby microsatellite loci, the age of the deletion was estimated at only about 700 years old (Stephens et al. 1998) and was conjectured to result in resistance to the plague (Stephens et al. 1998) or smallpox (Galvani & Slatkin 2003). However, Hummel et al. (2005) examined a sample of 2900-year-old Bronze Age skeletons and found that the frequency of the deletion (11.8%) was at similar levels as that found in a modern German sample (9.2%). If the *CCR5-Δ32* mutant provided protection from the plague, then one would predict that the frequency of this allele would be reduced in plague victims compared with a control group. Hummel et al. (2005) also found no evidence of a reduction in deletion frequency in a sample of fourteenth-century plague victims (14.3%) when compared with a contemporaneous sample of famine victims (12.5%).

Sabeti et al. (2005) used human genomic diversity data to suggest that aspects of the *CCR5-Δ32* data, including linkage disequilibrium, are not significantly different from other variants at the locus or throughout the human genome. They also used an improved genetic map of closely linked microsatellite loci and 32 SNP (single nucleotide polymorphism) markers and estimated deletion age to be 5075 (3150–7800, 95% confidence interval), consistent with a date in the Bronze Age.

These studies use two approaches that hopefully will become more widespread in other situations, ancient DNA and detailed genomic data. First, dating the deletion to at least 3000 years ago with the Bronze Age samples showed that the deletion was present long before plague was an important human pathogen and likely before smallpox was widespread. Nonetheless, this variant was probably adaptive in conferring

resistance to general infectious diseases that activate the immune system response provided by CCR5.

CCR5-Δ32 may be unusual for a selected variant because it results in a nonfunctional allele with a selective advantage. Usually loss of gene function would be under purifying selection, but because HIV and other pathogens use CCR5 as a gateway to initiate infection, loss of function appears advantageous. In red-capped mangabeys, there is a 24-bp deletion at CCR5 that has reached a frequency of 87% (Chen et al. 1998), and CCR5 is silenced altogether in sooty mangabeys, the natural hosts of SIV (simian immunodeficiency virus) (Veazey et al. 2003). In other words, without any major cost to resistance (de Silva & Stumpf 2004) that would result in a balanced selection in the presence of such a pathogen, adaptive selection could eventually result in fixation of *CCR5-Δ32*.

Malaria-resistant variants in humans. Malaria kills more than one million children each year in Africa alone and is the strongest selective pressure in recent human history (Kwiatkowski 2005). As a result, selective protection from malaria by sickle cell, thalassemia, G6PD, Duffy, and many other host variants provide some of the clearest examples of adaptive variation and diversifying selection for pathogen resistance. Genomic studies have demonstrated that selection for malarial resistance is strong, up to 10%, and that variants conferring resistance to malaria are recent (Hamblin et al. 2002, Ohashi et al. 2004, Saunders et al. 2005, Wood et al. 2005), generally less than 5000 years old, consistent with the proposed timing of malaria as an important human disease. Often the resistant variants are in different populations, probably owing in part to their recent independent mutation origin. However, Williams et al. (2005) provided evidence of negative epistasis between thalassemia and sickle cell and suggested that because there is no known cost to α^+-thalassemia, this is a potential explanation for the failure of the thalassemia variant to increase or become fixed in African populations.

Sickle cell hemoglobin provides the classic example of balanced polymorphism, with heterozygotes having a greater resistance to malaria than homozygotes, but sickle cell homozygotes suffer a cost from sickle cell anemia. A third allele *C* at this locus, which has a different single amino acid substitution at the same amino acid position, is in substantial frequency in several west African populations and has been shown to confer higher protection to malaria than sickle cell (Modiano et al. 2001). With estimated relative fitness from this data set, Hedrick (2004) showed that if *C* is introduced by mutation or gene flow, it will always increase and eventually become fixed, although the rate of increase of *C* is a function of the initial frequency of *S*. This outcome occurs primarily because genotype *CC* has the highest estimated relative fitness of any genotype. Overall, it appears that *C* has been slowly increasing in this west African population because of the presence of *S* and that now it is poised to rapidly increase to a high frequency within the next 50 generations, eliminating allele *S* and sickle cell anemia and going to fixation.

***R* (disease-resistant) genes in plants.** In recent years, there has been extensive research examining *R* (disease-resistant) genes in plants (Bergelson et al. 2001, Mauricio

et al. 2003, Meyers et al. 2005, Rose et al. 2004, Xiao et al. 2004), which has provided evidence for balancing selection. For example, Stahl et al. (1999) showed that resistance and susceptibility in the highly selfing plant *Arabidopsis thaliana* to the bacterial pathogen *Pseudomonas* is the result of molecular variation at the gene *Rpm1*. In a worldwide survey of *A. thaliana* accessions, they found that all susceptible plants were genotype *rr* and that all resistant plants were *RR*, except for one resistant accession from Kazakhstan that was heterozygous *Rr*. The overall frequency of the resistant *R* allele was estimated to be 0.52 throughout the range of the species. A fitness cost for the presence of this resistance allele in the absence of pathogens has been shown (Tian et al. 2003), providing a counter to the advantage of this allele for disease resistance and an explanation for the polymorphism of this allele. In general, researchers believe that to maintain polymorphism in a gene-for-gene system there needs to be a cost to the resistant host allele and a cost for the virulent pathogen allele, or they will go to fixation.

Allozyme Variation

Diversifying selection was often given as an important basis for the maintenance of genetic variation at allozymes, and discussion of these data was included in my previous reviews. Eanes (1999) provided an extensive review and evaluation of most of the subsequent data and the well-known case studies (see also Schmidt & Rand 2001, Storz & Dubach 2004, Véliz et al. 2004, Watt et al. 2003). Studies have continued to examine functional differences in allozyme molecules and, more recently, the amino acid basis for allozyme alleles (for a summary of these data for *Drosophila melanogaster* allozymes, see Eanes 1999). Recently, analysis of North American latitudinal clines for a number of allozymes in *D. melanogaster* showed that the derived allele is the one increasing with latitude, consistent with the model of an ancestral African species adapting to temperate climates (Sezgin et al. 2004).

Perhaps the most studied allozyme example is variation in the *Adh* locus in *D. melanogaster*, which provided the first detailed examination of DNA variation for an allozyme polymorphism (Kreitman 1983). But even for this extensively studied and cited example, there are still questions about the agent of selection, the selective importance of differences between the *F* (Fast) and *S* (Slow) variants other than the amino acid substitution, and the selective interaction of different loci or sites (Eanes 1999). Further, examination of DNA sequence data illustrated that the *Adh F* haplotypes had much less variation than the *S* haplotypes and showed little divergence from them (Kreitman 1983). As a result, it appears that the *F* haplotypes have increased in frequency only recently (the *Adh* polymorphism is also absent in closely related species).

Examination of *S* haplotypes in samples from Zimbabwe *D. melanogaster* (Begun et al. 1999) demonstrated that there was as much variation within this group as in the worldwide sample (Kreitman 1983) that included *F* haplotypes (see also Veuille et al. 1998). In other words, the excess of variation seen around the amino acid difference between the *F* and *S* haplotypes (Kreitman & Hudson 1991) may have predated the generation and the increase of the *F* haplotypes. Surprisingly, this instance of high,

localized variation correlated with a known functional polymorphism remains the only such example in *D. melanogaster*. This suggests either that there are few such balanced polymorphisms in this species or that recombination generally has obscured this signal of balancing selection.

THEORETICAL MODELS

The first theoretical paper that showed that diversifying selection over different habitats could maintain genetic polymorphism was that of Levene (1953). The important and continuing impact of Levene's letter to the editors in *American Naturalist*, which was just over two pages long, is illustrated by its citation nearly 600 times (**Figure 4**), starting with a number of citations in the influential 1955 volume of the Cold Springs Harbor Symposium on Quantitative Biology on "Population Genetics: The Nature and Causes of Genetic Variability in Populations." The number of citations continued to increase over time, but in the late 1980s and early 1990s, there was a dip in citation number. In contemporary literature, Levene's paper continues to be widely cited, with an average of 20 citations per year over the past decade. However, the type of citations has changed, with many early manuscripts extending the theory developed by Levene and later manuscripts stating that this type of selection is a potentially important explanation for the observation of genetic variation in a given species.

As an introduction to this section, I discuss the intuition of the evolutionary biologists that first considered diversifying selection and maintenance of polymorphism. In 1990, I wrote to Howard Levene regarding his short paper on polymorphism when there is variable selection in space (Levene 1953). In his response, Levene wrote that

> Dobzhansky asked me during a conversation whether polymorphism would be easier to achieve in a divided population. His intuitive feeling was that it would. When I told him the results in the letter he was very pleased, and said, "Fine, go ahead and send it

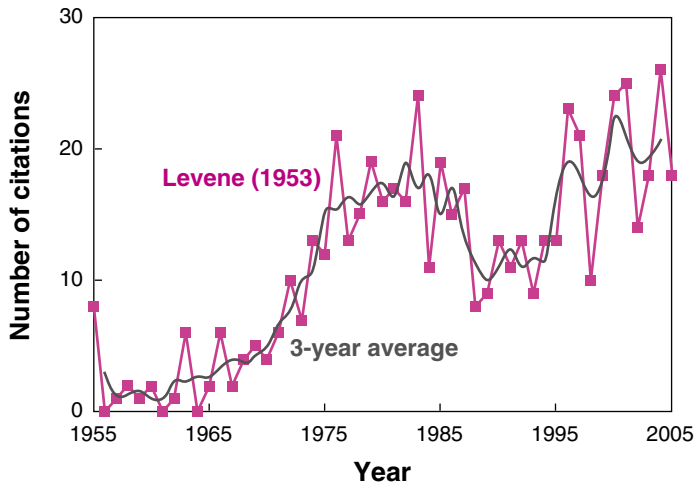

Figure 4
The number of annual citations for the classic article by Levene (1953) (*magenta squares*) over the past 50 years and the three-year running average of citation number (*dark gray line*).

> as a letter. But of course, you will now go into it in detail." I doubted I would and said the only really interesting question was how about selection differing by time instead of location. But then that was done by Everett Dempster. I don't remember what the other model with variable population size was and obviously I never did anything with it.

In addition, in the summary of Levene (1953), he stated that

> The model proposed is obviously not realistic: however, if it is modified by supposing that individuals move preferentially to niches they are better fitted for, or that there is a tendency for mating to occur within a niche rather than at random over the whole population, conditions will be more favorable for equilibrium, so that in a sense we are considering the worst possible case.

From the theoretical models developed since Levene's, we know that diversifying selection over either time or space generally does result in broader conditions for maintenance of polymorphism than when fitnesses are constant over environments. However, the conditions are generally more robust when there is spatial variation in selection rather than temporal variation in selection (Frank & Slatkin 1990, Hedrick 1986, Hedrick et al. 1976). Intuitively, if the environment varies over time—for example, one year is wet and the next year is dry—then every individual must endure every different environment whether or not they are genetically adapted to it. However, if the environment varies over space—for example, one area is wet and another dry—then only part of the population encounters a particular environment at a given time. In other words, when there is spatial variation in selection, some genotypes and a proportion of their descendants that are well adapted in a particular environment may not even encounter environments to which they are not adapted.

It is again important to emphasize that variable selection over space or time is not sufficient for the maintenance of polymorphism (Hedrick 1986, Levene 1953) because of the often-stated assumption that genotype-environment interaction will result in maintenance of genetic variation. Further, when there are more than two alleles and/or more than one locus and multiple environments, the conditions are not necessarily more robust.

For example, Prout & Savolainen (1996) examined the potential for stable polymorphism in the leafhopper, *Mocydia crocea*, using the fitness estimates of six leafhopper morphs on 20 host plants (Müller 1987). Exploring different starting frequencies of the gametes involved in determining the phenotypes, they found that variation at one gene was always fixed, while a two-allele polymorphism was maintained at the other locus. In other words, the conclusion that variation in fitness values of different leafhopper morphs on different host plants could explain the genetic variation (Müller 1987) does not appear to be true. However, they did find that each allele was favored on at least one host plant, and if gene flow was small between host plant species, then there would be a global polymorphism for all alleles.

For quantitative characters, the conditions for maintenance of genetic variation vary with genotype-environment interaction and sometimes do not result in the maintenance of variation (Via & Lande 1987), whereas other models do (Gillespie & Turelli

1989). Because of space constraints, I do not examine the maintenance of quantitative genetic variation by diversifying selection (see Byers 2005, Turelli & Barton 2004).

Spatial Models

Levene (1953) introduced the first theoretical model for examining the impact of diversifying selection in space. With random mating (and soft selection), the conditions that Levene derived depend on the harmonic mean of the heterozygotes being larger than the harmonic mean of the homozygotes. When there is limited gene flow and/or habitat selection, this further increases the proportion of the population that escapes the environment to which it is not adapted.

One of the best examples of spatial structure in the environment resulting in maintenance of genetic polymorphism is in the common bacterium *Pseudomonas fluorescens*, which evolves rapidly under novel environmental conditions and generates a variety of mutants (Rainey & Travisano 1998). In a homogeneous environment (constantly shaken microcosm), the original morph, smooth, is the only detectable one. But in a spatially heterogeneous environment (no shaking of microcosm) at least three morphs, smooth, wrinkly-spreader, and fuzzy-spreader, were found in substantial frequency in all replicate microcosms. Further, transfer of cultures from the heterogeneous environment to the homogeneous environment resulted in loss of about three quarters of the variation in just two weeks, and subsequent transfer back to the heterogeneous environment quickly restored the diversity level. Additional study of this system has examined the evolution of fitness trade-offs for these mutants, the genetic basis of the mutants, and other aspects of the system (Brockhurst et al. 2004, MacLean et al. 2004, Spiers et al. 2002).

When there is limited gene flow and differential selection between populations, the situation may become somewhat complicated (Hedrick 1995a, Kawecki & Ebert 2004, Lenormand 2002). As an example, on several islands in Lake Erie, the water snake, *Nerodia sipedon*, has both banded forms, as found on the mainland, and gray, unbanded forms thought to be a balance between selection against the banded forms on flat limestone rocks of the island because of bird predation and gene flow to the island from the banded mainland populations. The inheritance of banding appears to be determined primarily by variation at a single locus (King 1993), with the banded form dominant over the unbanded. King & Lawson (1995) estimated that the selective advantage (s) of unbanded individuals on the islands was between 0.11 and 0.28, and from variation in frequencies of allozyme alleles among the islands, they obtained an estimate of gene flow of $m = 0.01$.

Using these values of s and assuming that $m = 0.01$, the expected allele frequency of the unbanded allele on the islands can be calculated (Hedrick 2005). If $s = 0.11$, then there is an unstable equilibrium at 0.101 and a stable one at 0.890, and if $s = 0.28$, then there is an unstable equilibrium at 0.037 and a stable one at 0.953. For both levels of selection, the stable equilibrium frequencies are somewhat higher than the frequencies observed [0.53 to 0.86 on different islands, with a mean of 0.73 (King & Lawson 1995)]. This difference may be because s is lower or m higher than estimated, or perhaps the populations have not yet come to an equilibrium state. Overall, this

case study illustrates how the various evolutionary factors can be estimated and then put in a theoretical context to explain the observed patterns of genetic variation. It also illustrates that even for this relatively simple situation, the findings may be complicated.

Genotype-specific habitat selection such that individuals prefer niches in which they have higher fitness results in broader theoretical conditions for a polymorphism (Hedrick 1990). Further, estimates of average habitat selection (Hedrick 1990) based on *Drosophila* habitat choice data also provided much broader conditions for polymorphism. A single gene appears responsible for the polymorphism in bill size in the African finch *Pyrenestes* (Smith 1993), and, in this case, bill size and feeding preference are correlated, suggesting that the maintenance of genetic variation in this random mating population is related to habitat selection. In addition, sex-dependent habitat selection by itself can sometimes enhance the maintenance of polymorphism (Hedrick 1993). Recently, Ravigné et al. (2004) evaluated protected polymorphism for habitat selection under different models of population regulation.

Temporal Models

The first considerations of the maintenance of polymorphism from temporally variable selection were by Dempster (1955) and Haldane & Jayakar (1963). The conditions for an equilibrium for two alleles in diploids were that the geometric mean fitness of the heterozygote needs to be larger than the geometric mean fitness of the homozygotes (Haldane & Jayakar 1963), and the same conditions were shown to apply to multiple alleles (Turelli 1981). Even though the conditions for a stable polymorphism in an infinite population are unrelated to environmental pattern (Gillespie 1973), the distribution of allele frequencies is strongly affected by the autocorrelation between subsequent environments (Hedrick 1976). Recently, a temporal selection approach was used to model maintenance of multiple alleles at a MHC locus in which multiple pathogens were present or absent in different generations (Hedrick 2002).

Although individuals generally cannot avoid unfavorable environments when selection varies in time, some organisms may be able to avoid an environment for which they are not adapted because they can exist in a life stage that does not encounter the effect of the environment. For example, some plants have extensive seed pools, and the seeds that do not germinate do not experience many of the environmental effects encountered by the seeds that do germinate. Likewise, insects or other animals that undergo diapause can avoid environments encountered by individuals that develop without diapause. Such models have been investigated in an ecological context to determine their influence on the coexistence of competing species. This storage effect allows each of two competing species to survive environments for which they are not adapted by having a life-history stage that avoids the unfavorable environment.

These ecological models have been extended to show that the conditions for maintenance of genetic variation can be greater in fluctuating environments in which there is the opportunity for genotypes to escape unfavorable environments via the storage mechanism (Ellner & Hairston 1994, Hedrick 1995b). An example of temporal selection variation and seed dormancy is provided by flower color variation in a small

annual plant of the Mohave Desert, *Linanthus parryae*. This variation was the focus of debate among early evolutionary geneticists about what factors were important in determining the pattern of genetic variation and whether it was consistent with genetic drift (Epling & Dobzhansky 1942, Wright 1943) or explained by natural selection (Epling et al. 1960). Schemske & Bierzychudek (2001) reexamined this classic example in a polymorphic population where the frequency of plants with blue flowers ranged from 9% to 15.9%. For the four years with lower seed numbers (1988, 1989, 1992, and 1993), blue-flowered plants had many more seeds than did white-flowered plants, whereas for the three years with higher seed numbers, the opposite was true.

Turelli et al. (2001) developed theory to explain this situation. They determined the conditions for a stable polymorphism with variable selection and variable contributions to the seed bank and showed that a genotype whose arithmetic and geometric means are both less than unity can persist if its relative fitness is greater than unity in years with high reproduction (high contribution to the seed bank). In fact, they found that the higher fitness of the blue-flowered plants is balanced by a lower contribution of blue-flowered plants to the seed bank.

TESTS FOR BALANCING SELECTION

In the past few years, with the availability of multiple genome sequences for a number of organisms (particularly in humans), there has been an intensive search for genomic regions exhibiting a signal of adaptive (positive Darwinian) selection (Akey et al. 2004, Bustamante et al. 2005, Nielsen et al. 2005, Wang et al. 2006). Many of the regions identified have undergone a selective sweep because of favorable directional selection, as indicated by the signal of a high nonsynonymous to synonymous substitution ratio, or by low genetic variation and/or high linkage disequilibrium in closely linked regions. There appear to be many genomic regions with a history of adaptive selection, but only a small proportion of these indicate a genetic signal consistent with balancing selection. However, the smaller region thought to exhibit a balancing selection signal, compared with a selective sweep, may make detection beyond the resolution of most genomic scans. Further, alleles identified under directional selection may be part of balancing selection at a gene, and further examination of specific alleles may be necessary to clarify the nature of selection (Wang et al. 2006).

Recent studies in plants indicate that many loci reflect balancing or positive selection, although this may be an overestimate because demographic effects may not be accounted for and the examined loci are not a random sample (Wright & Gaut 2004). Genomic regions of *Arabidopsis thaliana* with high levels of intraspecific variability were specifically examined by Cork & Purugganan (2005), who identified several loci with other signals of balancing selection. Of course, identifying the basis of the fitness and phenotypic differences between the genotypes for these genes is necessary to understand their adaptive effects.

The MHC genes have become a paradigm for balancing selection, so much so that neutralists have often used them as an example. Garrigan & Hedrick (2003) examined the evidence for balancing selection at the MHC and for heuristic reasons divided the evidence as that in the current generation, that over the history of populations

(recent past), and that over the history of species (distant past). Evidence in the current generation is produced in that generation and can be lost in a generation, given that selection is not present. The generation of a balancing selection signal in the recent past is primarily determined by selection in combination with factors such as genetic drift, gene flow, and recombination and is generated or lost over tens to thousands of generations, depending on the influence of these factors. The signal of selection in the distant past is primarily determined by mutation and takes many thousands or millions of generations to generate or to lose (Garrigan & Hedrick 2003). Although the extent of evidence is less for other loci than it is for MHC genes, this division based on timescale and evolutionary factors is useful in evaluating evidence for balancing selection.

Current Generation

Identifying balancing selection in the current generation has been the classical approach, and it uses deviations from Hardy-Weinberg proportions, Mendelian proportions, or random mating proportions, as well as associations of specific genotypes and fitness when exposed to a given environment. As an illustration of the last phenomenon, Arkush et al. (2002) examined survival of endangered winter-run chinook salmon from known MHC genetic matings (one parent was heterozygous and the other homozygous) when exposed to three different important salmonid pathogens. For example, for the 10 families, each with 60 progeny, exposed to the infectious hematopoietic necrosis virus, 245 of 299 (81.9%) heterozygotes survived, and 228 out of 305 (74.8%) homozygotes survived. The estimated selection against homozygotes was 8.8%.

Recent Past

Since the development of the neutral theory nearly 40 years ago, a number of tests for evidence of balancing selection in the recent past have been developed. These include the Ewens-Watterson test (Ewens 1972, Watterson 1978) of allele frequency distribution, more linkage disequilibrium and less genetic variation in the particular region than for neutral markers, and geographic differentiation different from that found for neutral markers. For example, **Table 1** gives the estimate of F, the Hardy-Weinberg level of homozygosity for *HLA-A* for 12 different human populations (Garrigan & Hedrick 2003). Using the Ewens-Watterson test, three of these populations have less Hardy-Weinberg homozygosity than expected under neutrality, given the sample size and number of alleles.

Lewontin & Krakauer (1973) developed a test to examine the variation over populations for given loci. In theory, all loci undergo similar effects of genetic drift and gene flow, producing an expected variance over populations, whereas differential selection over populations increases this expected variance, and the same balancing selection over populations decreases this variance. In recent years, versions of this test have been used to identify outlier loci, that is, loci that have a significantly higher (or lower) F_{ST} than expected and therefore may be selectively important (for reviews, see Beaumont 2005, Luikart et al. 2003, Storz 2005).

Table 1 Ewens-Watterson's homozygosity *F* and Tajima's *D* statistics, and their probabilities (significant values are given in boldface), calculated for a global sample of *HLA-A* sequences, given the observed sample size (2*N*) and observed number of alleles (*k*) (Garrigan & Hedrick 2003)

Population	2*N*	*k*	*F*	Prob (*F*)	*D*	Prob (*D*)
Ainu	100	9	0.182	0.06	3.143	<**0.01**
Australian	367	7	0.327	0.24	3.736	<**0.01**
Chinese	298	22	0.140	0.48	2.356	**0.01**
French	248	20	0.162	0.60	2.440	<**0.01**
Havasupai	244	3	0.401	**0.03**	4.049	<**0.01**
Kagui	100	4	0.356	0.09	3.254	<**0.01**
Molucca	52	7	0.214	0.09	2.514	<**0.01**
Omani	236	27	0.100	0.42	2.498	<**0.01**
PNG-Lowland	94	6	0.336	0.32	2.447	<**0.01**
PNG-Highland	188	5	0.634	0.75	1.013	0.13
Zapotec	137	6	0.235	**0.02**	1.976	**0.01**
Zulu	200	23	0.069	<**0.01**	2.441	<**0.01**
Global	2164	45	0.126	0.73	3.691	<**0.01**

Distant Past

Finally, for evidence of balancing selection that may be from the distant past, tests used to detect these signals (Bamshad & Wooding 2003) are more recent and are based on the distribution of sequenced alleles and/or the level of sequence variability, such as the tests of Tajima (1989), Fu & Li (1993), and Fay & Wu (2000), or the comparative sequence divergence and/or variability between different classes of mutation, such as the ratios of nonsynonymous to synonymous substitutions and the tests of Hudson et al. (1987) and McDonald & Kreitman (1991). For example, Tajima's *D* was calculated in the same populations for which we examined *F* (**Table 1**), and 11 of 12 populations examined are significant using Tajima's test (Garrigan & Hedrick 2003). This significance occurs because Tajima's test specifically examines the extent of sequence divergence between the alleles, and the *HLA-A* alleles are very divergent from each other, whereas the Ewens-Watterson test does not incorporate sequence data. As a result, the sequence-based test of Tajima has much greater statistical power than does the allele frequency–based Ewens-Watterson test.

One of the most widely used sequence-based tests is that of McDonald & Kreitman (1991), a simple statistical test to examine synonymous and nonsynonymous sites in the coding region of a gene within and between species. If the observed variation is neutral, then the rate of substitution between species and the amount of variation within species are both a function of the mutation rate, and the ratio of nonsynonymous (replacement) to synonymous (silent) fixed differences between species should be the same as the ratio of nonsynonymous to synonymous polymorphisms within species.

Table 2 The number of nonsynonymous (N) and synonymous (S) substitutions for fixed (F) differences between species and polymorphism (P) within species, and the ratio N/S, for *Adh* (McDonald & Kreitman 1991) and *G6pd* (Eanes et al. 1993) in *Drosophila* and for *G6pd* (Verrelli et al. 2002) and *HLA-A* (Garrigan & Hedrick 2003) in humans and chimpanzees

	Drosophila				Human			
	Adh		*G6pd*		*G6pd*		*HLA-A*	
	F	**P**	**F**	**P**	**F**	**P**	**F**	**P**
N	7	2	21	2	0	5	0	76
S	17	42	26	36	44	23	0	49
Ratio	0.41	0.05	0.81	0.06	0.00	0.28	—	1.61

McDonald & Kreitman (1991) applied the test to data from the coding region of *Adh* from *Drosophila* species (**Table 2**), and the ratio of nonsynonymous to synonymous fixed differences was $7/17 = 0.41$, whereas the ratio of nonsynonymous to synonymous polymorphisms was only $2/42 = 0.05$. They suggested that the excess of nonsynonymous substitutions results from fixation of selectively advantageous mutations [see a more extreme situation in **Table 2** for the *G6pd* gene in *Drosophila* (Eanes et al. 1993)]. **Table 2** also gives data for two human genes, *G6pd* and *HLA-B*, known to be under positive selection. Although the pattern for the human *G6pd* gene suggested strong, historical, purifying selection, polymorphic nonsynonymous variants are in high frequency, contrary to the prediction of low frequency under purifying selection (Verrelli et al. 2002). For *HLA-B* (and three other *HLA* genes), an extreme pattern was found, reflective of very strong balancing selection (Garrigan & Hedrick 2003). That is, there were more nonsynonymous polymorphic sites than polymorphic synonymous sites, the polymorphic nonsynonymous variants are often in intermediate frequency, and there were no fixed differences between humans and chimpanzees, either for nonsynonymous or synonymous variants.

If balancing selection maintains variation for a long period, then the same polymorphism may be present in different related species. This phenomenon, known as trans-species polymorphism (Klein 1987), is a common feature for MHC genes (Garrigan & Hedrick 2003, Klein et al. 1998), and, as a result, often the most similar MHC sequence is not in the same species but in a related species. A dramatic illustration of the long-term impact of balancing selection is in artiodactyls (sheep, goats, cows, etc.), where balancing selection appears to have maintained allelic lineages for over 20 million years (Gutiérrez-Espeleta et al. 2001). Takahata (1990) showed that balancing selection results in a phylogenetic pattern similar to that of neutrality but on a much longer timescale [see discussion of self-incompatibility alleles in plants that have similar patterns in Hughes (1999) and Castric & Vekemans (2004)].

How common is trans-species polymorphism for other loci? An initial screen of sequences from humans and chimps found very little trans-species polymorphism (Asthana et al. 2004), and there are very few other documented examples (Hughes

1999, Wuif et al. 2004). In other words, balancing selection generally is not strong enough, nor does it act long enough to maintain polymorphism over species.

CONCLUSIONS

Recent genetic information has provided details on the basis of some of the examples of selection in heterogeneous environments. The next few years should provide many more molecular details of known examples and provide potentially new candidate loci to be examined further. There appear to be many loci exhibiting the signal of adaptive directional selection from genomic scans, but overall the proportion of loci where polymorphism is maintained by environmental heterogeneity is low. Future investigations should provide a general picture of how selection has shaped the history of different parts of the genome and indicate genes for further detailed investigation of selection in heterogeneous environments.

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Volume 37, 2006

Contents

Indexes