Chapter 9

Evolutionary Processes in Insects

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9.1 INTRODUCTION

Insect ecology is becoming inextricably bound with the study of the evolution and population genetics of insects. The diversity and variety of insect species, in both numbers of individuals and numbers of habitats exploited, emerges from a study of insect ecology and inevitably leads to the question of how and why. Insects comprise about 80% of the described animals in the world; over 900,000 described species as contrasted to 60,000 Chordata. The incredible diversity in form and function exhibited by insects invites enthusiasm, amazement, and sometimes, awe.

What are the attributes of insects that have contributed to their evolutionary success as measured by numbers of species, numbers of individuals, and numbers of habitats exploited? The Insecta represent one of the most complex arrays of evolutionary experimentation among eukaryotic organisms. Insects exhibit metameric segmentation with appendages capable of modification, and this, coupled with the mechanical advantages of an exoskeleton, has been exploited in diverse ways resulting in evolutionary novelty. Wings promoted widespread dispersal and escape and increased the range of feeding and breeding opportunities. A most intriguing adaptation is complete metamorphosis in higher insects, which has opened up a variety of habitat and food possibilities in which immature forms and adults often exploit radically different habitats and thus avoid competition. Insects tend to be small, have a high reproductive rate, a short generation time, diverse modes of dispersal, and diverse genetic systems. All of these attributes, coupled with their fantastic diversity, makes insects highly desirable experimental organisms for the study of evolutionary processes. It is not surprising, therefore, that insects such as Drosophila, Tribolium, Habrobracon, and others have contributed fundamentally to our understanding of ecology, genetics, behavior, and evolutionary theory.

Evolution by natural selection is an elegantly simple principle first proposed by Darwin (1859). Only two conditions are necessary for evolution to occur: reproduction and hereditary variation such that it influences the success of reproduction. So, evolution is the result of differential reproduction. As a result of natural selection, the most fit and best adapted individuals survive to reproduce and thus transmit their genes to the next generation. Although the principle is simple, the processes involved in adaptive evolution are complex and are treated in the following sections.

9.2 MATERIAL BASIS FOR EVOLUTION—GENETIC VARIATION AND REPRODUCTION

Fundamental to understanding the evolutionary process in insects is an appreciation of the underlying genetic basis for adaptation. Ultimately, the raw material on which natural selection acts is genetic variation, generated and maintained in a variety of ways. We first consider the origin and types of variation and then examine how it is molded by selection in the course of adaptation.

9.2.1 The Origin of Genetic Variability

Adaptation and evolutionary change ultimately arise by action of natural selection and stochastic events on genetic variability. What is the nature of this variability and how is it expressed? To understand the role genetic variation plays in the adaptive process, we must understand the genetic material itself.

9.2.1.1 The Nature of the Genetic Material. In recent years our concept of what constitutes a gene has changed rapidly and is still not resolved. Recent advances in molecular biology have revealed that the genetic material in eukaryotes is far more complex and diverse in its structure, organization, and function than previously imagined. Part of the genome consists of unique nucleotide sequences that probably encode for most of the genes eventually translated into enzymatic and nonenzymatic proteins. Other nucleotide sequences, which code for a protein or ribosomal RNA that is produced in large amounts, may be repeated many times. Nucleotide sequences that give rise to a diffusible product are called structural genes. In the case of a protein-producing locus, one structural gene encompasses one polypeptide unit. This polypeptide may represent a functional protein or serve as a subunit incorporated into a more complicated protein molecule (Lewin 1980). Regulatory genes control timing and rate of structural gene expression. Regulatory genes may or may not produce a gene product. Even when a gene product is produced, it may not be translated into a protein but serves as a nuclear RNA molecule of special function in gene expression that never finds its way into the cytoplasm. Unraveling the structure and function of the regulatory process is now a most active area of research.

Unique nucleotide sequences coding for proteins may constitute a relatively small part of the total DNA in eukaryotes. The rest, sometimes collectively called heterochromatin, may represent up to 80–90% of the genome. Heterochromatin is made up of highly reiterated, short to moderately long repetitive sequences. Some sequences may be repeated millions of times and dispersed throughout the chromosomes in discrete blocks at specific sites. The genetic role of heterochromatin is complex and poorly understood. At one time heterochromatin was regarded as inert and of little importance in genetic expression. Indeed, some molecular geneticists still regard most of it as "parasitic," or "selfish," DNA with no function other than its own replication and survival. Today we know that it is directly involved in such diverse processes as chromosome pairing, recombination, chromosome rearrangement, gene regulation and developmental pro-
gramming, speciation, and macroevolution (Bonner 1982), with the list of function and evolutionary role increasing as new discoveries are made.

**Extrachromosomal genes** represent a fourth class that reside outside the nucleus as circular DNA in eukaryotic organelles such as mitochondria and chloroplasts. In many respects these genes resemble those of prokaryotes, and there is convincing evidence that they represent highly modified symbiotic microorganisms acquired during early stages of eukaryotic evolution (Margulis 1981). Characterization of these genes is now advanced. Because of their small size and the ease with which they can be isolated and their DNA sequence, they are becoming increasingly important as tools in a wide range of molecular evolutionary studies ranging from tracing lineages and polygenetic relationships among taxa by restriction enzyme mapping to establishing rates of speciation (Brown 1981).

### 9.2.1.2 The Organization and Function of Structural Genes

Structural genes in eukaryotes are similar to genes in prokaryotes only in the general pattern of their expression. That is, the DNA code for protein is transcribed to an RNA copy of messenger RNA (m-RNA). This m-RNA is carried out of the nucleus and translated on ribosomes while interacting with different transfer RNAs (t-RNA) that order amino acids into a specific sequence along the m-RNA molecule. Eukaryote genes, however, are now known to be quite different from those found in prokaryotes in organization, regulation, process, and order on the chromosome (Lewin 1980).

Eukaryotic structural genes frequently consist of far more nucleotides than are ultimately translated into protein. The transcribed nucleotide sequences that code for protein (exons) may be interrupted by a sequence of transcribed, but untranslated, sequences of nucleotides called introns which are enzymatically excised before translation. The functional role of introns is unclear, but they have been implicated in recombination, chromosome rearrangements, genetic control of cell differentiation during embryogenesis, and other cellular and immunological processes. They may also facilitate reshuffling of exons to create new genes (Gilbert 1978). A gene coding for a polypeptide unit also includes long stretches of nucleotide sequences at either end that do not produce a gene product.

A recent startling discovery is the presence of a previously unrecognized class of DNA sequence called a **transposon**. This special DNA sequence, sometimes referred to as a “jumping gene,” was first described in corn by McClintock (1951). Transposons, under certain conditions, can jump from one chromosome to another and may be a major mechanism of chromosomal mutations and rearrangements. They may not only cause mutations but also alter the pattern of gene expression and evolution (Bonner 1982).

Regulation of eukaryote genes may be influenced by the presence of histone and nonhistone proteins coupled with the chromosome. Also, gene control may be altered by diminution, amplification, rearrangement, and modification or modulation through transcriptional, posttranscriptional, and translational control (Brown 1981). Indirect evidence suggests that such proteins (not present in prokaryotes) are somehow involved in regulating gene expression, but their mode of action is not clear. Although we lack a clear understanding of how gene regulation works in eukaryotes, it is clear that eukaryotes have evolved an extremely flexible regulatory apparatus. Without such flexibility the complex organ systems and way of life of higher organisms, particularly animals, could not have evolved. This flexibility also provides many avenues of adaptation to cope with new environmental situations.

### 9.2.1.3 How is the Genetic Material Changed?

If genetic variation is the raw material for evolution, how does it arise? By the genetic material we include both structural and regulatory genes as well as the various kinds of heterochromatic DNA. Changes in chromosomal and extrachromosomal DNA or mutations fall into two categories: those that result in the alteration of a single nucleotide or point mutation and chromosome mutations that involve a change in a gene segment or large blocks of DNA (Table 9.1). The effects on fitness of point and chromosome mutations vary greatly. Some may be essentially neutral while others are lethal. Point mutations of structural and control genes, as well as heterochromatin, may result in a limited change in expression of one gene and possibly genes with which it interacts. Chromosomal mutations, however, can result in more extensive reorganization of the genome that alters the pattern of gene expression over many loci simultaneously (Bush 1981). Chromosome rearrangements thus appear to be of prime importance in implementing major adaptive shifts and evolutionary novelty (White 1978). Almost all species, even those most recently

<table>
<thead>
<tr>
<th>Type of mutation in eukaryote nuclear DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A.</strong> Point or allelic mutations causing alterations in:</td>
</tr>
<tr>
<td>1. Structural genes (exons or transcription units) specifying protein</td>
</tr>
<tr>
<td>2. “Operator” genes adjoined a structural gene</td>
</tr>
<tr>
<td>3. “Control” genes</td>
</tr>
<tr>
<td>4. Introns</td>
</tr>
<tr>
<td>5. “Heterochromatin”</td>
</tr>
<tr>
<td><strong>B.</strong> Chromosome mutations (involving a gene segment or large blocks of DNA):</td>
</tr>
<tr>
<td>1. Changes in amount of DNA</td>
</tr>
<tr>
<td>a. duplication and deletion of structural, operator, and control genes</td>
</tr>
<tr>
<td>b. addition and loss of heterochromatin</td>
</tr>
<tr>
<td>c. additions or deletions of whole chromosomes (aneuploidy) or complete chromosome sets (polyplody)</td>
</tr>
<tr>
<td>2. Alteration of gene arrangement within a chromosome (inversions)</td>
</tr>
<tr>
<td>3. Shift or exchange of one or more genes or gene segments between non-homologous chromosomes (translocations)</td>
</tr>
</tbody>
</table>
evolved, differ in the karyotype and overall genetic architecture in some unique way.

9.2.2 A Basis for Evolution—Sex and Reproduction

Although mutations are the raw material on which natural selection acts, genetic variation is affected by several other processes such as recombination, genetic drift, and inbreeding. Another factor of profound influence on the pattern of genetic variation is the mode of reproduction. Insects and other arthropods have evolved a most diverse array of reproductive mechanisms controlling or affecting the level of genetic variation.

9.2.2.1 Sexual Versus Asexual Reproduction. Most insects reproduce sexually and are diploid. Many diploid species, however, contain polyploid tissues (i.e., with more than two sets of chromosomes) or tissues with polytene chromosomes that consist of multiple copies of a chromosome united together in a single giant chromosome. Meiosis in diploid species results in production of haploid eggs and sperm. Mating commonly results in transfer of sperm to the female where it is stored and released to fertilize eggs as they pass out the genital tract. In such species recombination occurs each generation by a variety of mechanisms including crossing over, random assortment of chromosomes during meiosis, and by recombining male and female chromosome sets at fertilization.

Parthenogenesis, development of eggs without fertilization, occurs in several forms in insects and has different consequences with respect to genetic variation. Arrhenotoky (haplo-diploidy) is the parthenogenetic production of haploid males, while fertilized diploid eggs develop into females. In this case genes are effectively dominant in the male and thus exposed to direct selection every generation. Concealed variability, except for attributes limited to the females, should be reduced vis-a-vis diplo-diploid species. Thelotoky is asexual reproduction by way of females only; sterile or fertile males are produced very rarely or not at all. Their genetic variability is predominantly limited to production of mutations, as recombination has been forfeited.

Several cytological mechanisms have been described for parthenogenetic arthropods (White 1973). These include apomictic parthenogenesis, in which the egg fails wholly, or in part, to undergo meiosis, so that no reduction in chromosome number results. Since apomictic reproduction is asexual, new gene combinations are unlikely except for new mutations. In autonotic parthenogenesis the egg undergoes meiosis, and the diploid condition is restored in a variety of ways (i.e., through fusion of two cleavage nuclei, two polar bodies, or a polar body with the egg pronucleus, Tremblay & Caltagirone 1973). Some genetic recombination may be possible.

Parthenogenesis may be facultative or obligatory within a species. It is obligatory in most thelytokous species—males are very rare or lacking. Cyclic parthenogenesis involves alternation of parthenogenetic and sexual generations. Aphids, cynipid wasps, and certain Diptera exhibit this and have very complex life cycles.

9.2.2.2 Mechanisms of Sex Determination. Sex determination in diploid species is usually the result of genetic balance, with the two sexes having different combinations of sex chromosomes. (Sex chromosomes are chromosomes containing the genetic information responsible for sexual differentiation in diploid organisms.) In many cases one of the sex chromosome homologues is visibly different. The sex that carries the heteromorphic chromosome is the heteromelic sex (i.e., XY or ZW), while the other sex is homogamic (XX or ZZ). In Drosophila a double dose of X chromosome determines females, while the autosomes contain male-determining genes. Thus, XY or XO Drosophila individuals are males because they have one X only; XXY or XX individuals are females, assuming the normal (diploid) complement of autosomal chromosomes is present.

The genic balance theory of sex determination is untenable with haplo-diploid species because the sex-determining chromosomes will have the same relative frequency in both sexes. Several hypotheses currently attempt to explain sex determination in haplo-diploid species (Crozier 1977), and other mechanisms of sex determination are being reviewed (White 1973). Environmental and physiological factors also affect sex determination and differentiation in insects (Bergerard 1972). The evolutionary implications of sexual reproduction have been discussed by Mittwoch (1967), Williams (1975), and Maynard Smith (1978, cf. Chapter 17).

9.2.2.3 Inbreeding, Genetic Drift, and Gene Flow. In sexually reproducing species population structure, patterns of dispersal, and stochastic factors can greatly influence gene frequency. The number and sex of breeding individuals, for instance, may result in radical departures from random mating. In parasitic Hymenoptera brother–sister matings are quite common. In other groups the breeding structure is highly polygamous and a few males may fertilize most of the females. Counts of individuals may be misleading in such cases, as the effective population number \( N_e \) may be smaller than the apparent numbers would indicate. Thus, in small populations if one male inseminates 100 females, \( N_e \) is slightly over 4 because it is proportional to the harmonic mean of \( N_m \) and \( N_f \) and is strongly influenced by the smaller values (see Crow & Kimura 1970). Under such conditions new mutations can be fixed rapidly through selection, inbreeding, and drift.

Effects of inbreeding and drift are often confused. The immediate outcome of inbreeding is to alter genotype frequencies but not gene frequencies. Thus, inbreeding tends to increase the frequency of homozygotes and reduce heterozygotes, each generation making it easier for selection to act on concealed homozygotes. Genetic drift, however, can change both geno-
type and gene frequencies. Genetic drift occurs primarily in small populations and results from genetic "sampling error." When the number of breeding individuals is small, some alleles may be lost or fixed in the population by chance alone, thus reducing genetic variability.

Population structure and the pattern of geographic distribution may also affect gene frequencies and the level of genetic variation within a species. Gene flow by immigration or emigration between populations may increase or decrease the number of alleles, with the magnitude and rate of change determined by the number of migrants involved, size of the population affected, and intensity of selection on new alleles (cf. Crow & Kimura 1970).

9.3 MEASURING GENETIC VARIATION—METHODS AND IMPORTANCE

We have seen that evolution by natural selection arises from genetic variation and sexual reproduction. Measuring and characterizing genetic variation and changes in natural populations has therefore taken on an ever more important role in understanding insect adaptation and evolution. Ideally, we need a cheap, rapid, and simple method for surveying allele frequencies of specific genes of adaptive importance. Thus, if we are studying the genetics of host plant selection by insects, we might want to examine genes controlling the chemoreceptors of different races of insects with differing responses to host plants. Although spectacular advances are being made in molecular genetics, it is not yet possible to provide nucleotide sequences for such genes from large numbers of individuals. For population studies we are more limited to what kinds of genes and gene products we can study.

9.3.1 Gel Electrophoresis

Major advances in our knowledge of the genetic structure of insect populations, however, have been made in recent years through use of a cheap and sensitive method, gel electrophoresis. Allelic forms of protein, differing by only a single amino acid, can often be identified if the substitution alters the overall electrical charge of the molecule. Over 80 proteins can now be routinely studied by gel electrophoresis in some animals. A major limitation of the technique is that only genes coding for soluble proteins can be studied. Membrane, chromosomal, and other proteins cannot be sampled. Furthermore, genetic variation in regulatory genes is not amenable to gel electrophoresis, and yet such genes are probably as important from an adaptive and evolutionary standpoint as structural genes. A third limitation stems from the fact that gel electrophoresis underestimates soluble protein variation, as only variants that alter the overall electric charge or config-

utation of the protein can be detected. Despite this, gel electrophoresis is superior to older methods used to measure genetic variation, which were time consuming and usually restricted to visible morphological characteristics and cytogenetic markers (e.g., chromosome rearrangements). More sophisticated biochemical techniques are also available which provide considerably more detailed information than one-dimensional gel electrophoresis. Two-dimensional electrophoresis, microcomplement fixation, restriction enzyme analysis, and DNA and protein sequencing, however, are more costly and time consuming. A detailed discussion of these methods and those used in insect systematics and biology is presented by Bush and Kitto (1978).

9.3.2 Chromosomal Organization

Cytogenetic and biochemical approaches to the study of chromosome structure have application in the study of genetic variation. Various cytogenetic methods have been developed to establish specific attributes such as chromosome number and form of the chromosome complement or karyotype of an organism. Cytogenetics has been the conventional way of looking at the gross anatomy of chromosomes since the turn of the century. Until about 1955, methods remained relatively unchanged and centered primarily on conventional DNA and chromatin stains and the light microscope. In recent years resolution has been greatly increased, and our knowledge of cytogenetic variation enhanced through use of the electron microscope and sophisticated staining techniques. New staining methods permit the cytogeneticist to resolve fine details of chromosome structure and to examine the distribution of heterochromatin and euchromatin. These methods make it possible to establish the presence of very small translocations and inversions, and thus to trace the pattern of karyotypic evolution with greater accuracy (Blackman et al. 1980, Schultz-Schaeffer 1980).

At the biochemical level gene sequencing methods are being used to map chromosome structure and establish the function of various parts of the eukaryotic chromosome, such as the centromere, satellite DNA, and unique sequence DNA. Molecular genetics, therefore, is an increasingly important tool. The methods employed [e.g., DNA sequencing, microcomplement fixation (MCF), DNA hybridization, or gel electrophoresis] will depend on what level of genetic delineation is required. Gel electrophoresis is currently the most inexpensive and effective method for routinely studying the genetic structure of populations, clines, races, species, and closely related genera. For evolutionary studies on higher categories, MCF and DNA hybridization are more appropriate. As techniques improve, DNA and protein sequencing may be used to resolve problems at all systematic levels. Only DNA sequencing offers the capability of establishing the fine details of genetic evolution at the molecular level of the gene, as nucleotide
sequences of specific genes and chromosome regions can be compared between species.

9.4 INTRASPECIFIC VARIATION

9.4.1 Genetic Variation Within and Between Insect Populations

The advent of gel electrophoresis and molecular genetics brought into question some long-held theories on the genetic structure of animal and plant populations. Most importantly, the level of genetic variation maintained in natural populations, at least in the structural genes amenable to study, is considerably higher than originally predicted (Lewontin 1974). This has divided population geneticists into two schools. On the one hand, the neutralsists feel that most variation expressed electrophoretically has a minimal effect on fitness and exists in a population because of a combination of factors such as mutation, finite population size, migration, and drift (Nei 1975), with selection playing only a minor role in maintaining different electrophoretic alleles. The opposite view is held by the selectionists who believe that most, if not all, electrophoretic variation is maintained by some form of “balancing selection” such as overdominance (superiority of the heterozygote), density-dependent selection, differential selection between two sexes or life stages, or variable selection over time and space in a heterogeneous environment (Futuyama 1979). Both groups assume that many deleterious substitutions are eliminated by natural selection and that advantageous mutations have a role in protein evolution (Wilson et al. 1977). The controversy is over how the polymorphisms are maintained, and this problem is yet to be resolved.

9.4.2 Factors Affecting Genetic Variation

Environmental heterogeneity is one attribute that has been given particular attention recently as an important factor in maintaining genetic polymorphism in natural populations. Because agricultural practices, such as planting monocultures with little genetic variation, often have profound effects on environmental heterogeneity and the distribution and importance of beneficial and pest insects, we examine a few examples (cf. review by Hedrick et al. 1976). The best examples come from associations between particular genotypes and environmental parameters. For instance, there are now several well-studied cases in both plants and animals that have demonstrated clinal, temporal, and density-dependent variation, as well as “area effects” due to environmental heterogeneity, in allele frequencies (Hedrick et al. 1976).

At another level genetic perturbation studies of natural populations of \textit{Drosophila} have shown that a suite of factors may affect allele frequencies at single loci or over all levels of genetic heterozygosity (Barker & East 1980). Changes in frequency of alcohol dehydrogenase alleles can also be initiated by altering concentrations of various alcohols in the larval food media (van Delden et al. 1975), and the proportion of heterozygous loci per individual can be raised or lowered by increasing or decreasing the number of environmental variables (Hedrick et al. 1976). Levels of heterozygosity can likewise affect survival. Populations heterozygous for the ADH locus appear to be better adapted to different environments than monomorphic ones (Bijlsma-Meeses & van Delden 1974). These experiments have important implications in mass-rearing insects for sterile insect releases, biological control, or pesticide screening. If levels of genetic variation are not maintained at near normal levels, characteristics may be radically altered. This evidence for adaptive changes supports the selectionist theory for maintenance of genetic variation in nature. The gene-environment relationship, however, is not a simple one and caution should be the rule in any attempt to establish cause-and-effect associations.

9.4.3 Chromosomal Variation

Chromosomal variation in natural animal populations arising from chromosome rearrangements (Table 9.1) has been extensively investigated and thoroughly reviewed by White (1973, 1978). \textit{Drosophila} has probably contributed more to this field than any other organism. \textit{Drosophila} (many Diptera) have giant banded polytene chromosomes in various tissues which permit identification of small chromosomal rearrangements, deletions, and duplications. Recent developments have also enhanced the resolution of chromosome studies of non-Dipteran species. With special chromosome banding techniques, many types of chromosome rearrangements can now be recognized (Blackman et al. 1980).

Certain chromosomal polymorphisms in populations are common in some organisms, but rare in others. Paracentric inversions (ones that do not include the centromere) in some \textit{Drosophila} and trimerotropin grasshopper species are widespread, while pericentric inversions, translocations, and changes in chromosome number are encountered in these insects only as rare mutants. In general, chromosomal polymorphisms are less common in other insect groups because they generally reduce the fecundity of heterozygotes. The conventional interpretation of why such chromosomal polymorphisms exist in certain populations is that they tie up blocks of coadapted gene complexes with superior fitness conferred on the heterozygotes (Mayr 1965). Although some laboratory experiments appear to support this view, alternative explanations have been offered (Bush 1982). It can be argued that the inversion polymorphisms found in \textit{Drosophila}
maintain a regulatory polymorphism rather than genetic variability or co-adapted complexes.

9.5 EVOLUTIONARY PROCESSES—GENETIC VARIATION BETWEEN SPECIES

9.5.1 Species and Speciation

Before we can consider genetic variation between species and its ecological and evolutionary implications, we must first understand what we mean by the term species. What constitutes a species and how species originate are actually one and the same question. How a species is defined will in turn profoundly affect the way an investigator interprets the way new species arise and vice versa. It is for this reason that so much controversy on this subject has been generated over the years. The conventional and most widely accepted definition of a species is based on the "biological species concept." According to Mayr (1963), "species are groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups." Although this is a handy operational definition for sexually reproducing eukaryotes, it cannot apply to asexual organisms and is difficult to use objectively in cases involving geographically isolated populations of closely related taxa. Speciation is the development of separate evolutionary lineages over time. If sympatric (e.g., their home ranges overlap), taxa must be sufficiently reproductively isolated from one another to indefinitely maintain separate identities. If they coexist in the same area yet retain separate gene pools, irrespective of how phenetically similar they might be, they may be recognized as species. When dealing with closely related, but allopatric (geographically isolated) taxa identification of species becomes more subjective. If reproductive isolation can be demonstrated by carefully controlled hybridization tests, species status can be conferred. However, when hybrids and backcrosses are normal, such cannot be inferred. Correct mate selection in nature may depend on certain premating isolating mechanisms such as habitat selection or some other ecological or behavioral cue not furnished in laboratory tests. Species that would never mate in nature may sometimes be artificially hybridized.

Strong morphological differences sometimes encountered between geographic isolates may also give a false impression. Evidence suggests that there is no direct cause-and-effect relationship between the level of genetic and morphological divergence in the degree of reproductive isolation (Wilson 1976). Therefore, taxonomic treatment of many allopatric demes is, at best, an educated guess. Whether two or more populations represent identifiable, independent evolutionary lineages will thus depend on the quality and extent of evidence mustered. Changes that result in divergence of separate lineages are attributed to complex interactions between spatial, temporal, ecological, stochastic, and genetic factors that affect epigenetic, physiological, behavioral, and morphological traits. Because the processes involved are diverse, and rather poorly understood, an unequivocal solution to the species' problem frequently voiced by evolutionary biologists is unlikely in the near future.

9.5.2 Methods for Measuring Genetic Differences Between Species

The most direct way to establish if related populations represent two or more species is to study their genetic structure. Because species are reproductively isolated from one another, it seems logical that a measure of genetic differentiation would resolve their status. Unfortunately, this is not necessarily the case. Ideally, we would like to compare nucleotide sequences of major sets of genes on a population basis, but this day has not yet arrived. Indirect methods such as gel electrophoresis, microcomplement fixation, restriction enzyme mapping, and polynucleotide hybridization techniques must therefore be employed. These can only give a rough approximation of the true variability, but they are sufficiently accurate for resolving many population/species problems. Gel electrophoresis is currently the method of choice for most population studies because it is simple and inexpensive. Genetic differences expressed electrophoretically can be used to establish the degree of genetic divergence between populations, species, and sometimes genera.

Several numerical approaches for quantifying electrophoretic data have been developed. Two commonly used are modifications of techniques developed by numerical systematists for analysis of morphological traits. One, called phenetics, involves comparisons of taxa on the basis of similarities (Sneath & Sokal 1973). Cladistics, on the other hand, establishes phylogenetic relationships on the basis of shared, derived traits (Wiley 1981). Both approaches have their advantages and adherents (Mayr 1981), but cladistic methods may be more appropriate for generating phylogenetic trees over those that employ phenetic methods (Wiley 1981).

The lesson from these studies on genetic variation in and between populations and species is that genetic differences expressed electrophoretically are not necessarily directly correlated with the degree of morphological differentiation. In certain groups large phenotypic differences may be accompanied by little or no measureable genetic divergence and vice versa. Allozymes, and the structural genes they represent, therefore appear to have little to do (at least directly) with evolution of the phenotypic traits used in systematics to establish taxonomic groupings.

Although cladistic methods are increasingly being applied to molecular data, most electrophoretic results in the past were analyzed by phenetic methods to establish differences between species. A similarity coefficient \( I \) is generated using the allelic variations expressed electrophoretically (Nei 1975). The coefficient of similarity may range from 1 (complete con-
cordance) to 0 (no alleles shared). Thus, if two populations have an $I$ value of 0.99, they share about 99% of their alleles. As the phenetic approach provides useful information on the overall genetic similarity between related taxa it is used here to illustrate some problems of relying too heavily on genetic data to establish species boundaries.

9.5.3 Examples of Genetic Differences Between Species

Much has been written on the origin and evolution of genetic differences between species, hard data on the degree and kind of genetic differences is meager. It is based almost entirely on electrophoretic studies and is reviewed by Nevo (1978), White (1978), and Ayala (1982). In insects comparative estimates are available only for *Drosophila*. This has led to an unbalanced view of the significance of genetic differences between species as this sample hardly reflects the genetic diversity known to occur in the Insecta. There are at least three problems in using electrophoretic data to estimate genetic distances between species. First, the DNA coding for structural genes that produce protein represents only about 10% of the total found in a eukaryote. As yet we have no way of establishing the level of variation or its function in the remaining 90%. A second source of error results from the nature of the genetic code. About 30% of the changes in structural DNA cause no amino acid sequence change in the resultant proteins. Finally, only about 40% of amino acid substitutions alter the overall charge of a protein and thus can be detected by electrophoretic methods.

Since *Drosophila* represents the most thoroughly analyzed group of insects, we will closely examine their pattern of genetic divergence. Dobzhansky et al. (1977) compared the genetic distance between taxa at different levels of evolutionary divergence in the *Drosophila willistoni* group, ranging from local populations to nonsibling species. Their findings are summarized in Table 9.2. Caution should be observed in interpreting these results, particularly with respect to subspecific and semispecies categories.

Both are operational taxonomic terms that are ill defined from a genetic and evolutionary standpoint. *Subspecies* represent geographic races of a species, each recognized on the basis of some phenotypic characteristic. As there is no test of sympatry, the decision to designate geographically isolated populations subspecies or species is usually made on purely subjective grounds and is open to interpretive error. In certain cases subspecies may represent distinct species. *Semispecies* are taxa on the borderline between subspecies and species. They qualify as species under some but not under other criteria. Again, classification is based on subjective interpretations. As pointed out by Bush (1975) and White (1978), most, if not all, the *willistoni* group semispecies could be regarded as distinct species. Each is characterized by its own set of unique chromosome inversions. Furthermore, several are sympatric. The Andean-Brazilian, Amazonian, and Interior populations coexist over a broad area. They do so without losing their genetic and phenotypic identity. They pass the test of sympathy and should be recognized as species. The reasons for conferring semispecies status apparently rests on the fact that under laboratory conditions they are not fully reproductively isolated. Such tests, as already noted, circumvent many natural barriers to gene flow and fail to account for those reproductively isolating mechanisms based on habitat selection and other environmental factors.

The genetic distances given in Table 9.2, therefore, do not provide an accurate picture of the kinds of genetic differences in structural genes one might necessarily expect between local populations, geographic races, and species. There is, in fact, mounting evidence from a variety of studies on vertebrates and invertebrates that genetic distances as measured by electrophoresis bear little if any direct relationship to the genetic changes involved in speciation (Nevo & Cleve 1978). The genetic similarity between some species of rodents, for instance, may be as high as 0.98, or as low as 0.63 between some local populations of the same species (Patton & Yang 1977). In some groups morphologically distinct species placed in separate families may have genetic distances no greater than those found between some sibling species (Wilson et al. 1977). For the reason behind the poor concordance between genetic divergence, morphological differentiation, and reproductive isolation, we must consider other types of genetic change such as those involved in altering patterns of gene regulation (Bush 1982).

### Table 9.2 Genetic identity and genetic distance between various levels of evolutionary divergence in the *Drosophila willistoni* group

<table>
<thead>
<tr>
<th>Level of divergence</th>
<th>Identity</th>
<th>Distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Local populations</td>
<td>0.970 ± 0.006</td>
<td>0.031 ± 0.007</td>
</tr>
<tr>
<td>B. Subspecies</td>
<td>0.795 ± 0.013</td>
<td>0.230 ± 0.016</td>
</tr>
<tr>
<td>C. Semispecies</td>
<td>0.798 ± 0.026</td>
<td>0.226 ± 0.033</td>
</tr>
<tr>
<td>D. Sibling species</td>
<td>0.563 ± 0.023</td>
<td>0.581 ± 0.039</td>
</tr>
<tr>
<td>E. Full species</td>
<td>0.352 ± 0.023</td>
<td>1.056 ± 0.068</td>
</tr>
</tbody>
</table>

*After Dobzhansky et al. (1977).*

9.5.4 Evolutionary Rates and Phylogenetic Trees

9.5.4.1 The Molecular Clock. Comparative biochemists have discovered that macromolecular sequences or proteins and nucleic acids evolve at relatively constant rates within major groups, and thus can be used as evolutionary clocks. Although the factors underlying this constancy are not understood, data indicate that variation in the rate of this clock within a given protein class is about twice that of a radioactive decay clock. This provides a better degree of accuracy than most fossil records available for
plants and animals. Wilson et al. (1977), review the evolutionary clock and its implications. The clock appears to be geared to years rather than generation time and provides an evolutionary time range for groups that lack or have a poor fossil record.

9.5.4.2 Molecular versus Morphological Phylogenies. Generally, there is a fair concordance between molecular- and morphologically based phylogenies. Whereas macromolecular evolution within a group appears to be rather constant over time, the rate of morphological evolution is more variable, proceeding through long periods of morphological stasis punctuated by rapid changes in morphology and way of life (Gould 1982). Furthermore, the rate and pattern of morphological change appear to differ greatly between major groups. Mammals have evolved much faster than some nonmammalian vertebrates such as amphibians. Phylogenies based strictly on macromolecular sequences, therefore, apparently reflect a better approximation of evolutionary relationships (Wilson et al. 1977) than those based strictly on morphological traits. Classifications based on morphological traits, on the other hand, do not necessarily provide close concordance with molecular-based, phylogenetic relationships. Because radical changes in morphology can occur over short periods of time, certain taxa that appear to be distantly related on morphological (or other biological) grounds may have diverged from one another fairly recently and be more closely related genetically than one of them is to a third species with which it is morphologically very similar, or even a sibling species.

9.5.4.3 Regulatory versus Structural Gene Evolution. Why does such a difference in the evolutionary pattern of morphological and genetic traits exist? Structural gene mutations appear to play a secondary role in adaptive radiation and speciation. Although these mutations may alter fitness of individual proteins, only a small minority appear to be involved in phenotypic change (Wilson et al. 1977). Major adaptive advances, such as acquisition of a novel metabolic activity or alteration of developmental pathways and ontogenetic development, appear to depend initially on changes in activity of rate-limiting proteins. Experimental studies on microbes and eukaryotes show that such changes are usually the result of mutations in control genes, in genes that exert control at levels other than transcription, or from chromosomal mutations that alter the arrangement of genes (Bonner 1982).

9.6 MODES OF SPECIATION IN INSECTS

There has been a quiet revolution over the past 10 years in our appreciation of how new species arise. As recently as 1963, with the publication of Mayr’s elegant Animal Species and Evolution, most evolutionary biologists became convinced that all sexually reproducing animals speciate only occurs as a result of genetic differences that accumulate during periods of complete geographic isolation (allopatric speciation—Table 9.3). This divergence must be sufficient to insulate that hybridization between populations, if it occurs, does not result in the breakdown of reproductive isolation. Recently several authors have questioned the universality of allopatric speciation in animals (Bush 1975, Endler 1977, White 1978, Templeton 1981). Alternative models have been proposed that do not require geographic isolation.

Speciation in animals may be operationally grouped into three categories based on presence or absence of geographic barriers to gene flow and mode of reproduction. These are allopatric, sympatric, and asexual speciation. Other schemes have been proposed by Endler (1977), White (1978), and Templeton (1981); they differ primarily in the way modes of nonallopatric speciation are grouped. We prefer to place them all under the general heading of sympatric speciation, with the exception of cases involving animals with asexual reproduction. While allopatric speciation requires complete geographic isolation of populations as a prerequisite, sympatric speciation occurs in situations where the ranges of two or more populations overlap in such a manner that interbreeding could occur unless prevented or greatly reduced by some form of genetically based isolating mechanism. The various forms of sympatric speciation listed in Table 9.3 represent different degrees and patterns of gene flow between diverging populations during speciation. They all share one thing: at no stage in speciation is the potential contact broken between parent and daughter populations.

9.6.1 Allopatric Speciation

Allopatric speciation was discussed in detail by Mayr (1963), Dobzhansky et al. (1977), and Carson (1975). Two types, the classical and founder principle models, have been proposed.

The classical model involves subdivision of a species into two or more large
geographically isolated populations. Divergence in such large populations occurs as a result of genetic changes incorporated in response to environmental changes that occur over time in the different geographic regions. First, the majority of these new alleles would exist as balanced polymorphisms for varying lengths of time. As such, they must be what Mayr called “good mixers,” they interact with other alleles throughout the genome without reducing average overall individual fitness. Mutations that appreciably reduce fitness of the heterozygote are not likely to be incorporated into the gene pool except under special conditions. Allopatric speciation by way of large, isolated populations is thus a slow process that requires long periods of isolation, and reproductive isolation would seldom be perfected when previously isolated populations reestablish contact.

A more rapid form of allopatric speciation, the founder principle, was proposed (Mayr 1963) that results from severe genetic bottlenecks in small, geographically isolated populations. Such populations may be founded by a small number of individuals that carry with them only a fraction of the genetic variation in the parent population. While the population is small, both chance and selection will play important roles in rapidly altering gene frequency. Mayr (1963) and Carson (1971) suggested that the combined effect of these two evolutionary forces will lead to a genetic revolution in the small, isolated population. Genetic divergence sufficient to insure reproductive isolation may then arise rapidly.

Both modes of allopatric speciation have apparently occurred throughout the animal and plant kingdoms. However, evidence in support of both types is circumstantial. Examples offered in their support are drawn from species that have already speciated, as no one has witnessed either type of speciation event in nature. Bush (1975), Endler (1977), and White (1978) noted that many cases offered as proof of the allopatric mode can be equally well explained by one of the sympatric modes discussed below.

9.6.2 Sympatric Speciation

9.6.2.1 Clinal and Area Effects. This form of sympatric speciation has been called parapatric speciation, and involves fragmentation of a species in an originally continuous cline or patchy environment into two or more species as a result of strong selection in the vicinity of an ecological boundary (White 1978). Both spatial segregation and spatial differentiation initiate the process and lead to evolution of isolating mechanisms between groups of geographically distinct, but contiguous, populations (Endler 1977). Three basic questions must be resolved in order to establish if it occurs in nature: (1) Can sharp genetic boundaries arise within a spatially and genetically continuous series of populations? (2) Can the resulting step clines give rise to hybrid zones? (3) Can reproductive isolation evolve in such zones? Direct field studies on plants and animals favor an affirmative answer to the first two questions, but as in the case of allopatric speciation there is only circumstantial evidence and theoretical arguments that the process of genetic differentiation in parapatric situations will eventually lead to speciation.

It is clear that contiguous populations occurring across a spatially abrupt environmental gradient or ecotone can diverge genetically to a considerable degree in a short time. Populations of Agrostis grasses, for instance, have become adapted to growing in contaminated soils of abandoned heavy metal mines in England. These mine-tilling races show marked differences in flowering time, amount of selfing, and other traits from the parent population on normal soil only inches away. Selection has favored evolution of a narrow hybrid zone resulting in greatly reduced gene flow between the two races. Of key importance in the Agrostis example is that the rate of gamete and seed dispersal is low and divergent selection in the two habitats is high enough to favor development of some reproductive isolation and a narrow, primary hybrid zone. Other examples and theoretical models are presented by Endler (1977) and White (1978). In no case has it been unequivocally demonstrated, however, that parapatric divergence has resulted in complete reproductive isolation. This question cannot be resolved until we have a better understanding of the relationship between gene flow and divergent selection across an ecotone. Current theoretical models, based on one or two locus situations, do not reflect the complicated, multilocus interaction occurring in natural populations. Such interactions can have unpredictable pleiotropic effects that could affect reproductive isolation and other adaptive processes. We must therefore withhold judgment on whether or not parapatric speciation can occur, although the evidence strongly suggests that it can.

9.6.2.2 Competitive Speciation. Another form of sympatric speciation involves divergence of two populations as a direct result of competitive interaction between genotypes adapted to different zones. It appears to be a common mode of speciation in several parasitic insect groups (Bush 1975) and possibly many other insects as well. Sympatric speciation in parasitic insects was reviewed by Price (1980).

Competitive speciation can occur rapidly and apparently with minimal gene changes. The best evidence supporting this comes from studies on insect pests that have formed new host races on introduced plants. The original host of the North American fly, Rhagoletis pomonella, is the hawthorn fruit (Crataegus spp.). Around 1860 it was found infesting apple, introduced over 200 years earlier. More recently, it established a population on sour cherry in a small area of Door County, Wisconsin. All three host races now coexist in this area. Each fly population has a different emergence time and pattern of host utilization, suggesting that the three races are biologically distinct. In these flies males and females meet and mate on the host fruit. In a series of papers Bush and colleagues (see Bush 1975 for summary and references) showed that host selection is determined mainly on the basis of chemical cues. Hybridization experiments with a related fly species
indicated that host selection and larval survival were controlled by only a few loci, suggesting that a host shift may occur through genetic changes that affect recognition of hosts, (perhaps involving chemoreception) and survival. Changes in host selection and survival genes could follow the gene-for-gene coevolved genetic system as seen for wheat and the Hessian fly (cf. Day 1974). Newly established host races have been observed in other insect pests (reviewed by Diehl & Bush 1983).

9.6.2.3 Stasipatric Speciation. The major differences between stasipatric speciation and clinal and area effect speciation is in the way reproductive isolation is attained. In the latter it arises by accumulation of genetic differences between parapatric populations across an ecotone. Stasipatric speciation is initiated by a chromosomal rearrangement that reduces fecundity when heterozygous, and when homozygous, produces a new adaptation. These mutations may arise anywhere within the range of the ancestral species (White 1978). This mode of speciation appears to be restricted to taxa that are subdivided into many relatively small, semisolated populations (Bush et al. 1977). As with parapatric speciation, stasipatric speciation is most common in organisms with low vagility or that have breeding systems that promote inbreeding. Small effective population size, coupled with inbreeding and drift, are a prerequisite for fixing chromosome rearrangements that reduce viability in the heterozygote (Lande 1979).

Stasipatric speciation may therefore be widespread in certain arthropods and other invertebrates. White (1978), who developed the concept of speciation by chromosome rearrangement to explain the origin and evolution of moribine grasshoppers of the genus Vadiotrenchella, discusses several striking cases in other grasshopper genera, phasmids, beetles, and isopods. Chromosome evolution and speciation have also been rapid in some parasitic Hymenoptera where inbreeding, including brother–sister mating, is widespread (Askew 1968, Goodpasture & Grissell 1975). Because this mode has probably played an important role in the evolution of new host races and sibling species of parasites, attempts to use parasitoids as biological control agents should be accompanied by careful screening at both the genetic and chromosomal level to establish the taxonomic status of each population sampled.

9.6.2.4 Polyploidy and Hybridization. These two factors have contributed significantly to multiplication of species in plants but are of little or no significance in animals. Polyploidy involves multiplication of complete sets of chromosomes. It can occur either as a result of autopolyploidy in which there is a multiplication of a chromosome set within a single individual or by allopolyploidy in which chromosome sets from two related species are joined as a result of the hybridization, then doubled during cell division by various mechanisms. Only the latter appears to be of significance in annual plants and possibly a few animal groups. The result is instantaneous speciation in which a new sexually reproducing species occurs in one gen-

eration. Interspecific hybridization has also been invoked to explain cases of speciation in both plants and animals in which no increase in chromosome number has been found. No example of polyploidy as a mechanism for speciation in sexually reproducing animals has been confirmed or supported.

9.6.2.5 Asexual Speciation. Many species of animals (and plants) reproduce asexually. Males are either nonexistent or rare and nonfunctional. Asexual reproduction occurs in almost all insect orders, including parasitic Hymenoptera, aphids, cockroaches, and flies. In an asexual species there is a common phenotype. There is no recombination through sexual reproduction, although a considerable amount of genetic and chromosomal variations may be accumulated by mutations. There are a variety of ways, including interspecific hybridization and polyploidy, that may give rise to an asexual taxa (see review by White 1978).

9.7 COEVOLUTION

Another aspect of insect evolution of major significance and interest to ecologists is coevolution, that is, reciprocal evolution arising from pressures that occur between different species which have close ecological relationships with one another (Ehrlich & Raven 1964, Gilbert & Raven 1975). One should remember that the coevolutionary process ultimately involves selective forces interacting between species at the genetic level. Thus, genomes of unrelated organisms are coevolving as an outcome of their close, and frequently, obligate association. The best examples at the genetic level are those worked out for host–parasite interactions. In insects the genetics of host race formation in the Hessian fly provides an interesting case. The larvae mine stems of wheat. Since its introduction into North America in the early 1800s, it has repeatedly formed races on new cultivars selected for resistance to its attack. Larvae usually die in resistant varieties but cause stunting in susceptible ones. Controlled test crosses and oviposition experiments have shown that resistance is conferred by a series of dominant genes in the wheat varieties and virulence (ability to overcome resistance) is the product of a series of recessive genes in the fly. Each cultivar has a unique resistant gene and can be attacked by only one race of the fly, one that is homozygous for a specific virulent gene (Stebbins et al. 1980). This gene-for-gene coevolved genetic system is typical of a range of host–parasite interactions (Day 1974).

In some cases host–parasite interactions are complicated by bacterial and viral symbionts. The genetic interactions involved, however, are not known. A few examples will suffice to illustrate the high degree of coevolutionary interaction. Many braconid and ichneumonid parasitoids, for instance, transmit highly specialized baculoviruses and other viroidlike particles when depositing eggs in their host (Edson et al. 1981). The viruses
appear to have an important commensal role with the parasitoid and somehow contribute to survival of parasitoid larva. Many insects also harbor highly host-specific, symbiotic bacteria, yeasts, and protozoans that are finely tuned to their hosts’ biochemistry (Buchner 1965). Cockroaches, for instance, have intracellular “bacteroids” that are transmitted in the egg cytoplasm. Roaches cannot survive without their symbiotes to provide several essential amino acids and other biochemicals (cf. Chapter 4).

Coevolution also may occur between plants and herbivores. Special morphological features (spines, pubescence) and chemicals (nutrient-poor sap, secondary plant substances, etc.) may lead to adaptation in the herbivore to circumvent the host’s defenses. Similar patterns of coevolution may also be found in certain predator-prey, plant-pollinator relationships, and mimicry complexes (Gilbert & Raven 1975). Coevolved systems are widespread in arthropods and frequently involve unrecognized relationships. There are probably few insects that do not harbor some viral or bacterial endosymbionts. The function of the vast majority of these is unknown but of great importance to understanding how certain kinds of adaptive variation are maintained in nature. Genetic aspects of host-parasite interactions, for instance, are essential to proper management of some insect pests. Certain aspects of community ecology can only be understood in the light of such closely interacting systems.

9.8 IMPLICATIONS FOR APPLIED ENTOMOLOGY

9.8.1 The Role of Genetic Variability

Extensive intra- and interpopulational genetic variability exists in arthropod populations and has important implications for applied entomology. Pest species may be able to respond rapidly to environmental changes, including those caused by man. A most obvious (and most economically important) example of rapid microevolutionary change is the development of resistance to pesticides.

Resistance is the development in a strain of the ability to tolerate toxicant levels that are lethal to most individuals in a normal population of the species. Natural tolerance is distinctly different; it is the preadapted tolerance shown by some species to some insecticides. Multiple resistance has sometimes been induced by simultaneous or successive exposure of a population to two or more insecticides. Development of pesticide resistance is a classic example of microevolution in which a toxic chemical acts as a selective agent to increase the frequency of the genes responsible for survival. Over 250 agricultural pest species and 100 species of medically important arthropods are known to be resistant to one or more major insecticides (Brown & Pal 1971). Resistance is a preadaptive trait, or arises de novo by mutation. There is no evidence that pesticides induce changes in the DNA responsible for the resistance. Mechanisms of resistance vary and include such attributes as activation and detoxification, reduced penetration and transport, larger capacity for storage or faster excretion, and reduced target-site sensitivity. Pesticide resistance may also involve changes in host or habitat preference, leading to “behavioral resistance.” The genetic basis of pesticide resistance has been widely studied (e.g., Crow 1957), but controversy still exists about the relative importance of monofactorial and multifactorial determinants due, in part, to problems of methodology. Inheritance of resistance was initially attributed to multiple genes, but studies soon indicated that DDT resistance was determined by a single major gene. Estimation of mortality at single “diagnostic” doses tends to result in the conclusion that the trait is a monofactorial one, whereas use of multiple doses tends to lead to a conclusion of polyfactorial inheritance. Crow emphasized that monofactorial inheritance is best proven by isolation of the factor through repeated backcrosses, coupled with selection. Resistance genes have been located on specific chromosomes in house flies, mosquitoes, and Drosophila melanogaster, and these studies have shown that resistance is usually determined by genes located on all the chromosomes, although major genes often account for the majority of the effect. Furthermore, several resistance mechanisms may coexist within a population, and variability between populations is common. Increased rate of detoxification and an altered site of action account for the majority of resistance mechanisms.

Devising methods to avoid or retard development of resistance is of high priority for pest management specialists. This can be achieved in several ways: reducing selection pressure through use of reduced rates and numbers of pesticide applications, and restricting applications to a small portion of the population. An alternative strategy of effecting complete mortality, so as to preclude selection, is operationally infeasible. However, development of resistance is unpredictable. Some species have undergone extensive selection and never developed resistance, presumably due to lack of appropriate preadaptive alleles.

Other types of resistance may develop in insect populations. The sterile insect technique requires that the insect can be mass reared, that sterility can be induced without adverse effects upon competitive ability and mating efficiency, and that population density estimates are available for low points in the population cycle to allow the calculation of the appropriate release ratio (Pal & Whitten 1974). Several components of a genetic control program are vulnerable to genetic responses by the target pest. Thus, insects that became able to reproduce asexually become invulnerable to autocidal techniques. Parthenogenesis is present in diverse insect groups and has developed many times (White, 1973). Moreover, parthenogenetic individuals occur at low, but regular rates in many sexually reproducing species, and it could be the ultimate escape from autocidal control programs.

Development of assortative mating, due to differences in behavior, diel
periodicity, pheromones, and so forth, may provide another escape from autodicial control. If divergent selection were sufficiently intense, distinct races might evolve rapidly. The propensity to mate varies within and between populations, and laboratory experiments may not expose this if one of the pest "species" is not included or if forced mating under laboratory conditions overcomes natural mating barriers.

There are other possibilities for evolutionary change to disrupt insect control programs. Control of populations by use of pheromone traps or "mating disruption" techniques could also fail due to genetic changes. Non-responsive insects could be selected for, particularly if visual and other cues are also critical for mating success. Genetic variability also exists for the use of, and metabolism of, juvenile hormone in insects (Templeton & Rankin 1978). Thus, these "third-generation" pesticides are vulnerable to development of resistance. The evolutionary potential of insects is generally underestimated by economic entomologists, but perhaps we can learn from the paradigm of resistance.

9.8.2 Genetic Variation and the Origin of New Biotypes

The appearance of new biotypes specializing on plant or animal hosts have frequently caused major problems. New host races have developed rapidly on a number of plants. The hawthorn fly, *Rhagoletis pomonella*, shifted to cultivars of apple in about 1860 and later to domestic sour cherries (Bush 1975). A similar pattern of host shifts has been noted in the codling moth which normally infests apple. This species has established distinct biotypes on walnuts and plums in California, with associated changes in host preference and numbers of generations (Phillips & Barnes 1975). The genetics of host race formation are discussed in Bush and Diehl (1982). Permanent shifts appear to require genetic changes in genes affecting host selection or recognition and survival. Little work has been done on the genetics of host selection, but there are several detailed studies on survival genes. For example, the Hessian fly has established genetically distinct strains on specific cultivars of wheat. The coevolved gene-for-gene system of resistance and virulence (survival) genes is now being used experimentally to exterminate cultivar races of the fly (Foster 1977). Other examples of race formation based on a shift in seasonal cycle are discussed by Tauber and Tauber (1981).

The speed with which insects can adapt to a new habitat or host is exemplified experimentally by Templeton (1979) who described a "genetic revolution" in *Drosophila mercatorum*. Given drastically different environments, a colonizing population can rapidly evolve a new set of balanced genes that are incompatible with the old gene complex. This genetic revolution can be manifested in terms of morphology, development, physiology, life history, and behavior. These alterations can be so drastic that "new species" can develop, complete with pre- or postmating isolating mechanisms in a very short space of time. In terms of pest control an exotic pest could evolve in unpredictable fashion in the new environment. Rapid shifts to new hosts can be expected as well. The genetic revolution may be due to genes that have fundamental regulatory roles as in juvenile hormone function or metabolism (Templeton & Rankin 1978). This revolution may depend upon a small number of loci, possibly as few as four.

The implications of host shifts and race formation for economic entomologists extend beyond changes in host plant choice by pest species. Biological control of weeds with phytophagous insects is predicated on the notion that host specificity will remain stable (Andres et al. 1976). Screening of host plant choices under insectary conditions is well known to overestimate host plant acceptability since the insect may never choose the host under field conditions. To date, we have no documented case in which basic host plant shifts have occurred after introduction of insects for weed control, due to the careful screening of such agents. However, it appears likely that such screening will never give total assurance of safety due to the unpredictable likelihood of a genetic change in the introduced insect.

9.8.3 Genetic Variability and Insect Colonization

9.8.3.1 Biological Control. Intra- and interspecific variability in insects considered for use in biological control innoculative release is widely recognized as potentially highly important. Establishment and efficiency of exotic natural enemies depends on many factors, including the initial sampling method of the population(s), rearing procedures, release strategies, and a host of other ecological and geographic variables. Since most foreign exploratory trips are of limited time and duration, it is unlikely that collections will include the full array of natural variation even though there is a strong tendency to include as many natural enemies as possible. Collections may undergo bottlenecks in the quarantine facility. Unfortunately, strategies for maintaining extensive genetic variability are not commonly practiced in biological control programs. Individual collections of a species should be taken at different sites, times of day, and seasons, if at all possible. These collections should be kept separate in quarantine and subsequent rearing, with each strain released individually. Collecting and maintaining several small colonies of different strains should maintain more variability than a single, pooled, large colony.

9.8.3.2 Genetic Problems in Mass-Rearing Programs. With the advent of sterile insect release methods for pest control, a new era in mass rearing of insects began. Suppression or eradication of a major pest species is achieved by flooding the population with infertile, but still sexually competitive, males mass reared in a factory. The technique was demonstrated first by eradicating the screwworm fly, *Cochliomyia hominivorax*, from Florida and the southeastern United States. Later, it was suppressed in Texas and
the southwest, and now attempts are being made to push it back to southern Mexico. A major difficulty has been in maintaining tight quality control over the factory-reared flies. Various factors such as larval diet, temperature fluctuations, and other factory conditions and practices can drastically reduce competitive ability. Most of these effects can be reversed, but if the cause goes unnoticed, selection alters the genetic structure of the factory population, and the effect may be irreversible, so that a new strain must be introduced. This has occurred on several occasions over the course of the screwworm eradication program, and it is instructive to look at this example in detail (Bush 1979).

Success of the program depends on the ability of released flies to compete with wild flies for mates. Although the program experienced stunning initial success, it ran into difficulties in Texas in 1972 when reported infestations jumped from 473 to over 95,000 and remained high in subsequent years, despite the fact that the number of sterile flies released rose from less than 2 billion per year at the start in 1962 to about 10 billion in 1974 (Bush 1978). Scientists at the screwworm facility suspected that factory conditions might be inadvertently selecting a strain of flies with reduced competitive ability. To ascertain if genetic drift or selection was occurring in the factory, genetic variation in factory and wild populations was studied. The objective was to see if the processes of colonization and factory rearing had any detectable genetic effect on the flies. Natural populations of the screwworm are low, ranging from 100 to 200 per mi²; thus, released flies must disperse and remain active at appropriate times if they are to find suitable living conditions and mate in competition with wild males. Therefore, the genetic study, using standard gel electrophoresis techniques, concentrated on glycolytic enzymes involved with flight, although others were examined as well.

One enzyme, α-glycerophosphate dehydrogenase (GDH) is of particular interest because something is known concerning the adaptive function of its allelic forms. This enzyme plays a key role in regulating energy flow in the flight muscle of insects during flight, as it governs transfer of reducing equivalents from cytoplasmic NADH to the mitochondrial electron-transport chain by way of the glycerol phosphate shuttle. The amount of GDH present in flight muscle greatly affects flying ability, and mutants which lack GDH cannot fly. Because of its central importance in regulating energy flow, allelic variants of this enzyme are under tight natural selection (cf. Bush et al. 1976 for details). Variation in these enzymes appears to be maintained by a combination of spatial and temporal patterns of environmental variation in temperature.

Upon electrophoretic analysis, all factory samples examined differed significantly from wild flies in the frequency of alleles at almost all the electrophoretically detectable loci examined. This was particularly true of loci controlling flight activity, such as GDH, which showed the most dramatic change (Bush et al. 1976). In the screwworm the GDH enzyme exists in two forms. Almost all factory flies were homozygous for electromorph GDH₂, while the enzyme of the wild form was extremely rare in wild Texas populations, which typically retained the alternate form, GDH₁. Therefore, the difference in GDH between wild and factory flies provided an important clue to the problem. Systematic sampling of a new factory strain was undertaken, from its introduction in the laboratory to its eventual adaptation to factory mass-rearing conditions. As soon as the new strain was introduced into the factory, GDH₂ began to increase in frequency and GDH₁ to decrease. Within six months, GDH₂ had become as common as in previous factory-adapted strains. But does the GDH₂ form of the enzyme affect flight activity?

Research on the function of the various forms of GDH in other insects, such as butterflies and fruit flies, suggested that each form of the enzyme functioned satisfactorily only within a specific, but different, temperature range (cf. Johnson 1974). To test whether the two forms of the GDH enzyme were affected by temperature, both forms were isolated and purified. It was then established that they did indeed have quite different temperature ranges within which they showed optimal activity (Kito et al. 1976). The factory-type enzyme (GDH₂) was less active in the temperature ranges experienced in nature in the morning when flies were active and mating. The high constant temperature used to speed development in the factory apparently exerted a strong selective force favoring GDH₄ over GDH₁ (Bush et al. 1976). This suggested that the competitive ability of the fly in nature would decrease as the frequency of GDH₂ increased because factory flies would have to cope with a wide temperature range in nature and individuals which lacked the GDH₁ enzyme simply could not fly as well at the appropriate time as their wild cousins.

Although the mating behavior of wild flies has never been observed in detail under natural conditions, studies on released factory flies suggest that at least part of the mating activity occurs in the air and possibly at specific sites which require normal flight and behavioral response (Guillot et al. 1978). Thus, the "lazy" factory males would be at a disadvantage competing for mates. In fact, a USDA team found that wild females were attracted to wounds from early morning to late afternoon. Factory females were not active until early afternoon. They apparently could not get their flight muscles operating for lack of sufficient energy. Most wild females therefore were probably inseminated by wild males before the factory flies become active enough for sexual activity. Also, the lazy flies might be subject to higher predation, further reducing their efficacy as biological control agents.

In 1977 the defective factory strain was eliminated and replaced by a new strain with the defective allele at low frequency or absent. Care was taken not to mix the old and new strains. Infestation levels that summer dropped to pre-1972 levels and, combined with adverse climatic conditions for the fly during the spring and summer, excellent control was regained.
in Texas, although difficulty is still being experienced elsewhere and further outbreaks have occurred. Recent cytogenetic and morphological studies also indicate that this fly may be subdivided into rather distinct geographic races, further complicating control efforts by the sterile insect release method (Richardson et al. 1982).

The problems encountered in 1972 could have been averted if the factory had had a sound quality control program to monitor factory populations for harmful genetic changes. Some effort had been made to measure effects of diet and other rearing conditions on fecundity, development time, longevity, and so on, but no tests were conducted to measure known adaptive, ecological, and behavioral traits pertinent to the fly’s survival in nature. Therefore, when the flies failed to perform, it was difficult to act because insufficient effort had been devoted to basic ecology and behavior, so that no one knew what might be causing the problem.

Although the screwworm example serves as a model, the effect of selection on several other species during mass rearing or laboratory colonization has been established or inferred for a diverse array of biological traits important to a pest control program. These include phototactic behavior and vision (Markow 1975, Goedenough et al. 1977), locomotory behavior (Chabora 1969), oviposition, premating, and mating patterns (Rossler 1975), and diapause (Hoy 1978). Work of Homyk and Sheppard (1977) exemplifies the ease with which behavior traits can be selected. They were able to recognize and establish 48 behaviorally different strains of *Drosophila melanogaster*.

**Evolutionary Processes in Insects**

Strain hybridization could be used for inundative releases of parasitoids or predators where establishment need not be permanent. Hybridization of standard insect strains could result in insects of uniform quality. Quality control in insectary rearing is a problem that has gained increased recognition as having both genetic and environmental components, and hybridization may provide one solution.

Selective breeding offers another method whereby beneficial insects may be genetically “improved” (Hoy 1976, 1979, Roush 1979). Artificial selection is the more controversial of the genetic improvement techniques suggested and has been demonstrably effective under field conditions with pesticide-resistant predators of spider mites (Hoy 1982, Hoy et al. 1983, Roush & Hoy 1981). Whether this success can be achieved with other biological control agents remains to be seen. The problem seems complex since a suite of desirable attributes must be maintained during the course of a laboratory selection program in which the beneficial insects are expected to be the effective agents. The mode of inheritance of relatively few desirable attributes are known, making selection inefficient. Sufficient genetic variability must be available to permit a response to selection. Some have suggested that after the release, “improved” strains will revert or be swamped genetically by the wild strain, thus, nullifying the extensive (and expensive) efforts of the program. Most of these problems might be reduced or eliminated if the problem is carefully defined (Hoy 1976, 1979). Most critics of genetic improvement are thinking about the genetic improvement of a parasitoid or predator that is to be released and permanently established in the environment. That is a difficult goal, which might have a limited success rate. However, “genetic improvement” may be feasible if more limited goals are sought. Not all attributes of the “wild” insect need be maintained, nor may they even be desirable. For example, obligate diapause in the silk worm is undesirable if two harvests per year are desired, and normal flight, dispersal, habitat finding, and fastidious food habits are undesirable under factory conditions. Parasitoids released into glass houses or field crops for temporary suppression of pests need not disperse, diapause, or find the appropriate habitat since they can be delivered to it. High fecundity, pesticide resistance, adequate sex ratios, and high parasitization or predation rates are desirable and are attributes that are amenable to selective breeding (Hoy 1976, 1979).

**9.8.4 Genetic Improvement of Beneficial Insects**

Genetic improvement of beneficial parasitoids and predators has been discussed for more than 60 years because of the successes achieved by plant and animal breeders and with silk worms and honey bees (Rothenbuhler 1979, Yokoyama 1973). A number of beneficial insect species have been targets of artificial selection programs (reviewed by Hoy 1976, Messenger et al. 1976). Genetic improvement of beneficial insects can be accomplished in several ways. One method suggested involves increasing the genetic variability of the beneficial species through laboratory hybridization of different strains, with the assumption that natural selection will effect a desired improvement after field release. Classical biological control, where limited numbers of an exotic species are introduced into new habitats to establish and effect control of a pest, is plagued with the problems of “adequate” sampling or securing of exotic populations in space and time, and with maintenance of the sometimes limited genetic variability achieved in foreign collections during quarantine processes, insectary rearing, and colonization. Thus, hybridization of strains, either before or after release, may be a way of increasing or maintaining sufficient genetic variability to obtain establishment and/or improve the efficacy of beneficial species (Hoy 1976, 1978).

**9.9 CONCLUSIONS**

Population and molecular genetics have made rapid advances in recent years, shedding new light on the process of adaptation and evolution. Contributions at the molecular level have been particularly rewarding with respect to improving our knowledge of how genes function and are organized on the chromosome. More sophisticated biochemical tools are also now available for examining the pattern of genetic variation at the protein
and nucleotide level. We are therefore beginning to perceive what constitutes the genetic machinery that programmes the development and other cellular functions that have long been viewed as a "black box" by population geneticists. Knowing how the genome is put together and how it works removed considerable uncertainty on how certain adaptations might evolve.

Although the molecular biologist may provide us with the mechanisms of gene action, the population biologist and naturalist will have the important responsibility of integrating this new genetic knowledge into a modern synthesis of evolutionary biology. The applied biologist also has much to gain from these advances as they open up many new roads to pest management and the development of resistance in crops and domestic animals.

REFERENCES


