

Invited Paper

Speciation: A Review of Concepts and Studies with Special Reference to the Lepidoptera

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PART I: Concepts

Introduction

Darwin introduced his concept of speciation through natural selection as the central process in evolution, not just as a process within populations of organisms but also as the ultimate origin of biological diversity. And while modern genetics has provided an understanding of the overall mechanism for evolution, from the molecular level to population biology, the genetic changes during speciation remain the subject of intense study and controversy.

The evolutionary significance of speciation for sexually reproducing organisms such as Lepidoptera is the origin of reproductive isolation, since only after the cessation of gene flow can related populations genetically diverge toward separate evolutionary fates. The genetics of speciation is thus a more narrowly defined topic than overall genetic differences among closely related species. Many points of debate have centered around this distinction between the genetics of speciation and the genetics of species differences (Templeton, 1982).

Three important and general findings in population biology over the last three decades relate to theories and models of speciation. First, sexually reproducing organisms contain surprisingly high levels of genetic variation within and among populations. This result calls into question early concepts of limits on population divergence which held that homogenizing gene flow and the supposed adaptive value of highly integrated gene systems must maintain similar gene frequencies among populations. Second, various parts of the genome evolve discordantly such that morphology, enzyme variation, and ecological adaptations, for example, may be poor predictors of reproductive isolation. Third, reproductive isolation is neither an intrinsic nor inevitable result of overall genetic divergence, but may arise only under special circumstances of population structure and selection.

These findings reveal the limitations of traditional taxonomy based solely on morphological criteria (Byers & LaFontaine, 1982; Collins, 1984; Gall & Sperling, 1980; Hafern timer, 1982; Lorkovic, 1985; Mayr & Ashlock, 1991; Oliver, 1972, 1978, 1979ab, 1980; Sperling, 1987; Wake, 1981; Zink, 1988), including male genitalia in Lepidoptera (Porter and Shapiro, 1990). As an expedient in classification and identification, Lepidoptera field guides customarily treat all species as discrete units of

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equal taxonomic rank, each with distinctive morphological and ecological characters. Evolutionarily "old" species may have accumulated many of these differences either before or after the origin of reproductive isolation, thus creating the impression that many classes of taxonomic characters change concordantly during the speciation process. The evolutionary biologist necessarily concentrates not on these so-called "good" species, but on taxa which lack discrete boundaries with related entities such as overlapping phenotypic variation or incomplete reproductive isolation - i.e. supposed examples of speciation in progress. Taxonomic decisions are especially difficult to make for allopatric populations whose reproductive isolation cannot be determined directly, or for morphologically distinct taxa which hybridize in nature. Assigning taxonomic rank in these cases should be secondary to collecting and evaluating evidence relating directly to genetic compatibility and reproductive isolation, and other factors regulating (or potentially regulating) gene exchange between the taxa.

A current trend in population biology is to view the component populations and demes of a species as genetically diverse, more or less independent ecological and evolutionary units (Murphy & Ehrlich, 1984). Speciation models must distinguish between the overall, inevitable tendency of separate populations of a given taxon to diverge genetically, and the origin and significance of reproductive incompatibility among related populations. Although this question is usually phrased in terms of the origin of "reproductive isolating mechanisms", this may not be (as discussed in section 3 below) the proper conceptual context for understanding speciation.

Practical difficulties arise because speciation cannot be studied directly. The rate of speciation in sexual animals is too slow to be observed and the complex interaction of population-level genetics with the natural environment prevents a realistic laboratory simulation of speciation. (Severe artificial selection on interbreeding populations in the lab has improved incipient reproductive isolation [Thoday & Gibson, 1962; Rice, 1985] but these experimental results are difficult to extrapolate to speciation in nature). Three basic approaches can be used to identify the genetic changes accompanying speciation, especially those concerned with reproductive compatibility among closely related taxa:

- 1.) We can study genetic differentiation among populations and recognized subspecies within a species. If we assume that such intraspecific differentiation represents incipient speciation we can extrapolate the changes accompanying speciation. This assumption is open to the criticism that speciation may be a rare and unique population genetic process. Arguments concerning the limitations of the subspecies concept (Arnold, 1983, 1985; Brittnacher et al., 1978; Forbes, 1954; Futuyma, 1986, ch. 4; Gillham, 1956; Hammond, 1985, 1990; Wilson & Brown, 1953) are a part of this criticism.

2). We can look back in time and catalog the observed differences among taxonomically very similar species, assuming that they represent the result of recent speciation. However, we cannot know if divergence in a given trait occurred before or after speciation. Comparisons can be made in methods 1) and 2) among populations with respect to population size, geographic distribution and degree of geographic isolation, rates of gene flow, ecological associations, etc. - factors affecting population genetic processes.

3). We can employ laboratory and natural hybridization to study the genetics of traditional taxonomic characters, life history traits, and ecological adaptations. Experimental hybridization is especially revealing because it is also a functional assay of reproductive and developmental compatibility of the parental taxa as expressed in the hybrid genome. Yet, only certain organisms-happily including many Lepidoptera-lend themselves to this kind of experimental manipulation. Unfortunately, perhaps in the quest for perfect specimens, experimental hybridization is often omitted from taxonomic studies, even when comparative material is reared in the lab. When hybrids are obtained, quantitative data on fertility, viability, developmental rates, and diapause should always be recorded.

I summarize in this paper major advances in speciation theory from various viewpoints. My purpose is not to evaluate this entire body of work; comprehensive reviews of speciation theory include Barton (1989); Barton and Charlesworth (1984); Bush (1975, 1982); Futuyma (1986); Otte and Endler (1989); Templeton (1981, 1982, 1989); other papers are cited in the text. With this background I will then discuss representative studies of speciation in Lepidoptera.

Lepidoptera have served as subjects for many important evolutionary studies (Vane-Wright & Ackery, 1984). Much of the underlying natural history literature is the result of amateur studies and I hope to encourage the continued contribution of amateurs, especially in mutually rewarding cooperation with professional biologists. I have endeavored to present this paper to a broad range of readership.

Species Concepts

1. The **Biological Species Concept** dates back to the synthesis by Dobzhansky (1937) of Darwinian theory, classical genetics, and mathematical models of population genetics into a genetic theory of evolution. As currently applied, the "biological species" (Dobzhansky, 1970; Mayr, 1963) is composed of populations contributing to a gene pool, united by actual or potential gene exchange, adapted to a unique range of ecological niches, and "protected" from disruptive interbreeding with related species by means of reproductive isolating mechanisms. This concept recognizes the considerable phenotypic and genetic variability of the populations comprising a species, in contrast to the "typological" or morphological species characterized by an idealized, uniform phenotype. Species

are thought to arise in allopatry, either through intervention of a physical barrier or by colonization, when cessation of gene exchange allows genetic divergence. According to this view, speciation is most likely when populations are small, such as newly founded populations at the periphery of a species range. Here the effects of genetic drift (reduced genetic variability due to random "sampling" of the parental gene pool) and inbreeding could produce novel genotypes in the founding population (Carson, 1968; Carson & Templeton, 1984; Mayr, 1982; Templeton, 1980). The interaction of these genotypes and their subsequent recombinants with a novel selection regime in a new environment may lead to a "genetic revolution" - a new, stable equilibrium in gene interaction - which may accompany a shift to a new ecological niche. Incipient reproductive isolation, such as altered mating times, may develop incidentally to these genetic and ecological adaptations.

Upon secondary sympatry, the two differentiated populations may interbreed but their hybrid offspring may be subvital, sterile or otherwise unfit. Overall genetic divergence in allopatry can reduce the fitness of hybrids by disrupting embryo and larval development, diapause, or adult development (postzygotic isolation). Selection would then favor the improvement of prezygotic (premating) isolating mechanisms which would reduce the gametic waste of interspecific matings. Selection cannot, of course, improve postzygotic isolation since hybrids are less fit. Morphologically distinct taxa which interbreed in nature do not challenge the Biological Species Concept but serve as examples of this final stage of speciation where reproductive isolation is perfected (Remington, 1985).

The origin of reproductive isolation accompanied by a period of genetic reorganization or "revolution" both stem from the concept of a highly integrated genome unique to each species. The gene pool of the biological species is assumed to be composed of an array of "coadapted gene complexes", groups of genes acting together in a highly coordinated manner and adapting the organism to its environment. This model is based partly on studies of chromosome inversions in natural populations of *Drosophila* (Dobzhansky, 1970, ch. 5,9; Lewontin, 1974, ch.3). Such inverted gene sequences (identified by characteristic banding patterns in polytene chromosomes) are thought to be of adaptive value because their frequency often varies in predictable geographic and seasonal patterns in specific populations. (Their true adaptive value is still to be determined.) The inversion acts to prevent crossing over and recombination in meiosis and is therefore thought to be favored by selection as a mechanism to maintain the gene complexes intact. This interpretation appears to corroborate the mathematical models of Wright (1931) who described the stable equilibrium of specific combinations of alleles for interacting genes. The allele frequencies for given loci in a population reflect the fitness that specific allele combinations confer; other populations in different ecological settings are characterized by different allele frequen-

cies and combinations. Unfavorable combinations reduce fitness and thus selection controls allele frequency by eliminating less fit genes and gene arrangements. In a small population selection and fortuitous gene frequency changes due to drift (or - at the extreme - local extinction and recolonization) can shift the population to more favorable gene combinations, or "adaptive peaks" in Wright's graphic depiction.

Intraspecific test crosses between *Drosophila* populations with differing inversion types have produced progeny with reduced fitness. By extension, interspecific lab hybrids are often subvital or barren presumably because of the disruption of their respective coadapted genomes. These observations provided the basis for the idea of the highly integrated gene pool unique to each species. Speciation would then necessarily require revolutionary changes to achieve a new set of harmonious gene complexes appropriate for new adaptations. Selection in turn would favor perfection of isolating mechanisms preventing disruptive interspecific hybridization. If this view of the genetics of speciation is true, one of the constraints on any "genetic revolution", and on overall speciation rates, must be the accompanying loss of fitness during such transitions. This is especially true for traits directly relating to reproduction, where independent gene systems in the two sexes control separate but compatible aspects of mate location and fertilization. Any mutation affecting mate recognition in one sex would probably be disruptive unless an unlikely complementary change occurred fortuitously in the opposite sex.

During the last two decades researchers have critically reexamined the Biological Species Concept, aided by computer modeling and mathematical analysis.

2. Criticism of the Biological Species Concept.

a. Gene flow and population structure.

The origin of species in allopatry remains a widely accepted model, both because biogeographic patterns of variation support it and because well established theory shows that even small rates of gene flow between populations can effectively prevent genetic divergence. Our understanding of the factors which maintain species integrity among separate populations has changed over the last three decades. In the Biological Species Concept gene flow and a highly coadapted genome tend to unify the gene pool. Ehrlich and Raven (1969) point to practical problems in testing the potential for gene exchange and question the assumption that gene flow rates among populations are high enough to prevent significant genetic differentiation. Populations are often widely separated and distance alone can act as a barrier to gene flow. They view species as genetic mosaics of variable populations whose relative reproductive isolation may be untestable. No one population can characterize a species and reproductive isolation may be poorly developed in certain groups, making taxonomic boundaries arbitrary. The population or deme is the

ecological and evolutionary unit, not the taxonomic species. Entire species do not evolve uniformly geographically nor synchronously (Ehrlich & Murphy, 1981; Murphy & Ehrlich, 1984).

Recent concepts (Endler, 1977; Slatkin, 1973, 1987; Templeton, 1980) de-emphasize the unifying effects of gene flow and a highly integrated genome. The genetic makeup of a given population is a function of not only the stabilizing effects of gene flow but also the opposing effects of mutation, genetic drift, and diversifying selection. In addition, the present genetic structure of a population reflects major historical events (e.g. glaciation, past periods of selection) affecting its present demography and genetic variability. The organization of genes within the genome is relevant to speciation processes (Templeton, 1982). Genes may occur as many loci throughout the genome, each with a small effect, or at the other extreme may exist as one major controlling gene with a few modifier loci. In a founder population genetic drift more likely would be important for a single large-effect gene than in the case of the polygene system.

Various classes of genes respond differently to selection. Thus, even in the absence of significant gene flow, geographic variation may be minimal for genes controlling critical developmental and metabolic pathways, mating and reproduction, and other characters under strong stabilizing selection. Other traits, such as morphology and life history traits, may exhibit abundant geographic variation. The adaptive value of much morphological variation - including that used to characterize subspecies - is often unclear, and while such characters may at first appear taxonomically useful, they may not be well correlated with overall genetic differentiation among populations within a species. The widespread view that subspecies are necessarily incipient species (e.g. Hammond 1985, 1990) is in general unfounded. As will be discussed below, reproductive isolation is likely to arise under special circumstances of population genetics.

In Lepidoptera examples of adaptive geographic variation include regional host plant specialization (Bowers, 1986; Fox and Morrow, 1981; Scriber, 1983), voltinism (Rabb, 1966; Waldbauer, 1978), polyphenism (Janzen, 1984; Shapiro, 1984ab), polymorphisms related to mimicry (Gilbert, 1983), etc. Unfortunately, the genetic basis for these adaptations is generally poorly known. Conversely, it has been difficult to establish the adaptive value of the considerable geographic variation in allozymes (Ehrlich & White, 1980; Johnson, 1976; Kingsolver & Wiernasz, 1991; McKechnie et al., 1975; Watt, 1968).

Some of the best studied examples of the adaptive value of geographic variation in life history traits are in the frog genus *Rana*. Moore (1957) revealed a north-south cline in genetic adaptations regulating larval development in response to water temperature in the *R. pipiens* complex (Leopard frogs). Experimental hybrid tadpoles showed distortions in body size and shape which increased as a function of geographic distance and difference in ambient water temperature experienced by the paren-

tal populations. This work eventually led to a systematic revision of the group (Sage & Selander, 1979 and cited refs.). In a similar study (Berven & Gill, 1983) found egg size, larval growth rates, and mature larva size to be critical adaptive variables, yet their relative genetic correlations and heritabilities varied within and among species as geographically separate populations were compared. In other words, the underlying gene systems were not just "fine tuned" in a clinal fashion, but were fundamentally reorganized in response to diverse environmental selection.

The evolutionary significance of these examples is that adaptive geographic variation among populations within a species is not qualitatively different from that distinguishing ecological and physiological characters among related species. Shifts to new adaptive modes or ecological niches can occur within species, even along continuous clines, and often without accompanying changes in gross morphology or reproductive compatibility.

b. Genetic revolutions and speciation in founding populations.

The early concept of a genetic revolution in a founding population leading to speciation has been revised by its advocates (Carson, 1982; Carson & Templeton, 1984; Mayr, 1982; Templeton, 1981, 1982). These authors promote the theory of rapid speciation in small isolated populations, but differ with respect to the number and kind of genes involved, and the type of selection acting on them. The Carson model of reorganization of polygene balances involves a relaxation of selection (during a phase of rapid population growth) allowing a major reorganization of the genome. Templeton describes the action of strong selection on a few major genes in favoring a shift to a novel Wrightian adaptive peak. Both authors cite the example of the Hawaiian *Drosophilids* where isolation of small founder populations by lava flows seems to be involved in the extraordinary adaptive radiation of these flies into more than 700 endemic species (Carson & Kaneshiro, 1976).

Barton (1989), Barton & Charlesworth (1984), and Felsenstein (1981) give critical, population genetic analyses of the restricted conditions under which genetic changes leading to reproductive isolation are actually likely to occur. Barton & Charlesworth (op.cit.) conclude that the probability of speciation during a single founder event is extremely low. Reduction of variability due to drift, which could promote a reorganization through the uniting of rare recessives, actually reduces the likelihood of shifts to new adaptive peaks. Templeton (1981, 1982) advocates the role of a few major genes in the origin of reproductive isolation, yet the substitution of a new allele in a single gene with major effects on reproduction will be especially opposed by selection. Conversely, the sequential change in many genes with smaller effects, as in the Carson model, is unlikely to occur quickly in a founder event. Barton & Charlesworth (op. cit.) support the model of a change in selection favoring

gradual genetic change over many loci which eventually results in reproductive isolation. While this process does not necessarily require a small, isolated population, novel selection regimes are in general more likely at the periphery of a species range, on islands, or in mountain ranges isolated by desert, etc.

The many surveys by biochemical geneticists have found abundant allozyme variations within and among populations, but have not found evidence for a genetic revolution. Allozyme types and frequencies vary progressively through a hierarchy of intraspecific populations, congeners, and related genera (Avisé, 1976). It may be generally true that most of the genetic differences (based on allozyme studies) between closely related species are present as polymorphisms within these species (Lewontin, 1974 ch.4).

c. Genetic changes during speciation.

Electrophoresis reveals variation in "structural" genes coding for metabolic enzymes, which are unlikely to relate directly to speciation. Electrophoretic studies are very useful in constructing phylogenies and in detecting gene exchange between populations (Geiger and Scholl, 1985; Geiger & Shapiro, 1986; Porter and Geiger, 1988), but show only correlation, not causation with regard to speciation.

The "regulatory" aspects of the genome controlling reproduction and development are more relevant to speciation but are also more difficult to study than allozyme variation, especially in organisms for which we lack gene sequence or linkage data. Experimental hybridization can reveal reproductive and developmental incompatibilities, but cannot reveal directly the underlying genetic basis (Collins, 1984; Haferník, 1982). By crossing members of geographically isolated populations of *Phyciodes*, Oliver (1972, 1978, 1979ab, 1980) demonstrated genetic incompatibilities (disrupted diapause and emergence schedules, etc.) within species which increased with distance of separation but differed only in degree from developmental problems seen in interspecific crosses. In assessing genetic differences between closely related species it is important to remember that some of the incompatibility seen in hybrids may be due to post-speciation genetic divergence rather than resulting from the speciation process. Geiger (1988) and Lorkovic (1986) discuss and debate the proper application of experimental hybridization and enzyme assays to taxonomic problems in Lepidoptera. Indices of enzyme similarity and hybrid compatibility are in general agreement in comparisons ranging from intergeneric to subspecific. Discrepancies may occur in specific cases near the species level. Parker et al. (1985) employed both hybridization and electrophoresis to demonstrate developmental incompatibilities among related Centrarchid fish.

The products of regulatory genes are likely to be relatively small molecules present in low concentrations, which regulate the action of other genes distributed throughout the genome (Britten & Davidson,

1969; Hedrick & McDonald, 1980). If such a system more accurately describes "coadapted gene complexes" than the tightly linked inversion sequences of the Biological Species Concept, this finding would bolster the criticisms of speciation through genetic drift in founder populations because changes at one or a few loci would have less effect in an interacting, distributed system of genes each with small effects. When many loci are involved new, favorable combinations are less likely to occur (see (b) above).

Studies with *Drosophila* are the most detailed examples of the genetic basis of reproductive isolation (Dobzhanski, 1970, p. 340 ff; Ehrman, 1962; Schafer, 1978; Sved, 1979). Typically, effective prezygotic isolation is based on the additive effect of many genes controlling a variety of physiological, morphological, and behavioral traits, none of which is effective by itself. In an important synthesis of published data, Coyne and Orr (1989a) analyzed pre- and postzygotic isolation in *Drosophila* hybrids with respect to genetic similarity (based on allozyme surveys) and sympatry vs. allopatry of given species pairs. They found a strong correlation between strength of prezygotic isolation and sympatry, which suggests the evolution of mating barriers due to selection against hybridization. Postzygotic isolation seemed to evolve in allopatry, increasing in severity with genetic distance, which is in turn an index of evolutionary time. The *Drosophila* seem to be an exception to the general lack of evidence in other animals for improved premating isolation in sympatry (see discussion in Section 3 below).

Coyne and Orr (1989b) relate their findings to the work of Charlesworth et al. (1987) on the occurrence of hybrid incompatibility in the heterogametic sex (Haldane's Rule); in Lepidoptera females are XY and sterility and inviability is almost always confined to hybrid females. In this model, beneficial recessive mutations arising during divergence in allopatry tend to accumulate faster on the sex chromosomes than on autosomes. But upon hybridization following secondary contact the expression of these genes is disrupted in the hybrid genome, and especially in the heterogametic sex where no dominant gene would be present to mask the recessive allele on the X chromosome. The Charlesworth model thus provides an explanation both for the origin of postzygotic isolation and for Haldane's Rule in hybrids between closely related species (Coyne & Orr, 1989b). There is recent evidence that key regulatory genes controlling the expression of a disparate collection of reproductive traits may reside on the sex chromosomes (Cardé & Baker, 1984; Charlesworth, et al., op. cit.; Grula & Taylor, 1980ab; Hagen, 1990; Taylor, 1972; Tuskes & Collins, 1981). Further research, perhaps employing new techniques in gene sequencing and mapping, will reveal if this phenomenon is a general mechanism in speciation.

Yet, in general we still lack a detailed knowledge of the genetic basis for traits associated with reproductive isolation (Lewontin, 1974). If an overall genetic revolution does not accompany speciation, how much

genetic divergence is necessary to achieve reproductive isolation? In closely related *Lepidoptera*, for example, how many genes control respective differences in wing pattern and associated courting behavior, or interspecific variation in pheromones and their time of release? How much geographic variation exists in genes controlling reproductive traits within nominal species? What are the factors involved in the origin and maintenance of this variation? Is this variation of the same order of magnitude as that found in allozymes, morphology, or ecological adaptations? Some geographic variation is known to occur in the component chemicals of pheromones, although in only a relatively few cases can differences be interpreted as antihybridization mechanisms (Cardé, 1987; Cardé & Baker, 1984). Rutowski (1984) reviews the role of sexual selection in the evolution of butterfly mating behavior.

3. The Recognition Species Concept.

Paterson (1986) formulated this concept in an attempt to correct perceived shortcomings in the Biological Species Concept. He defines species as "the most inclusive population of individual biparental organisms which share a common fertilization system". The Recognition Concept looks at "reproductive isolating mechanisms" from a different point of view. Traditionally, labeling traits involved in reproduction as isolating mechanisms implied that they evolved through natural selection for this adaptive "purpose". Paterson stresses that their origin is based on their adaptive value in identifying mates, regulating the initiation and full expression of courtship in both sexes, and achieving successful mating and insemination. In this context, any subsequent role such traits might play in isolating a gene pool from hybridization is irrelevant, especially if they arose in allopatry.

This theory of the origin of mate recognition traits has generally been acknowledged as useful and has been supported by the general failure to document the improvement of reproductive isolation in areas of sympatry between species, or in stable hybrid zones (Butlin, 1989; Spencer et al., 1986). See Endler (1989) and Endler & McClellan (1988) for a more general discussion of the role of mate recognition/isolation traits in evolution.

Other aspects of the concept have been extensively criticized (Coyne et al., 1988; Templeton, 1989). The major points of criticism can be summarized as follows: 1) The true adaptive origin of reproductive traits does not invalidate the role isolation plays in allowing species to evolve independently. 2) Paterson ignores the role postzygotic isolation plays in regulating gene exchange between taxonomic entities. Hybrid incompatibility is not an intellectual abstraction, as Paterson suggests, even if this unfitness arose from genetic divergence unrelated to selection favoring species recognition. Postzygotic incompatibility acts to stabilize genetic processes in hybrid zones by regulating gene exchange. 3) The Recognition Concept is not superior in correctly assigning species status since it

is burdened with many cases of geographic variation in prezygotic isolation (or recognition) traits within species. This criticism responds to the symmetrical argument by Paterson that many apparently distinct species lack effective prezygotic isolation and thus cannot be distinguished by isolation criteria.

4. Geographic clines and parapatric speciation.

Clines are formed when phenotypic characters vary over a geographic gradient. This kind of variation is well known for *Lepidoptera* and other organisms; many subspecies are connected to other populations through character clines. Most clines are thought to be the phenotypic response to environmental selection gradients varying geographically in intensity. Slatkin (1973) and Endler (1977) have analyzed clines in terms of formal, mathematical models. Clinal variation will be minimal when gene flow rates are high and selection is weak. Abrupt clines occur when gene flow is weak and selection gradients are steep. Endler (op. cit.) believes that gene flow rates have traditionally been overestimated and that most populations are only weakly connected by gene flow. Thus, strong selection can geographically differentiate a species. If critical genes adapting a population to its environment are closely linked to those controlling mate choice, natural selection could favor the origin of reproductive isolation if gene flow rates were below some critical value. This model is often referred to as parapatric speciation.

Endler (op. cit.) also demonstrates that in the absence of good biogeographic evidence this kind of primary differentiation would be indistinguishable from secondary contacts between previously isolated populations. The term "cline" is often used too casually in the *Lepidoptera* literature to refer to anecdotal examples of phenotypic variation. Many other phenomena cause taxonomic confusion by mimicking clines: mosaics of small populations where variation is due to founder effects rather than selection gradients; stable polymorphisms; narrow bands of sympatry between noninterbreeding but phenotypically very similar species; and hybrid zones where two taxa regarded as separate species are hybridizing. Critical analysis is required to distinguish these situations.

5. The Subspecies concept and its relevance to speciation models.

Subspecies are recognizably different geographic populations or sets of populations assigned formal taxonomic rank. The use of the subspecies category has proliferated in the taxonomy of *Lepidoptera*, partly because it is a catch-all for difficult problems in classification, but also because of the natural tendency of taxonomists to split intensely studied groups into new named entities. Many evolutionary biologists feel the naming of morphological subspecies is largely an arbitrary decision, which on the one hand may help catalog variation within a species, but on the other hand may actually mislead further study by implying a specific genetic status and by ignoring other significant patterns of divergence.

Four specific objections can be raised against the subspecies concept. First, there is no testable criterion to assign the subspecies rank, such as reproductive isolation as a test for species status. How different must a population be to be called a subspecies? Second, a subspecies may be a member of a clinal or mosaic array of variable populations. Geographic variation in several characters may not be congruent, making geographic limits for a subspecies quite arbitrary. Third, other populations undistinguished by phenotype may actually have diverged in more significant ways, such as adaptations to new hostplants, shifts in mating time, etc. Fourth, traditional morphological subspecies are not necessarily undergoing incipient speciation, even when isolated in unique environments. As has been discussed above, the origin of reproductive isolation is conservative and may require special circumstances of population genetics.

6. Hybrid Zones as natural experiments in speciation.

From the standpoint of the collector, hybrid zones are usually first detected as areas where two taxonomically distinguishable populations interbreed to produce a population of hybrids. With further analysis, hybrid zones are seen as narrow character clines maintained by hybrid unfitness. This definition is a practical way of distinguishing hybrid zones from intraspecific clines and clines or blend zones between recognized subspecies. Subspecies, by definition, should interbreed without loss of fitness, but the subspecies concept is arbitrary since the genetic and geographic boundaries of subspecies are themselves arbitrary. The distinction between hybrid zones and blend zones is predicated on knowledge of genetic compatibility; morphological analysis cannot reliably distinguish these phenomena nor determine if a zone is the result of primary or secondary intergradation (Endler, 1977; Section 4, above).

The structure of hybrid zones suggests a dynamic equilibrium between gene flow, which would tend to widen the zone, and hybrid unfitness which would tend to narrow the zone. Prezygotic isolation is either lacking or ineffective such that the two taxa cannot exist in sympatry without interbreeding. The two parental phenotypes are rarely found together in most zones unless hybrids are effectively sterile in both sexes. Studies of hybrid zones provide evidence against the concept of the origin or perfection of premating isolating mechanisms in response to hybridization (Butlin, 1989; Paterson, 1986; Spencer et al., 1986). The historical context of hybrid zones is difficult to determine, but many appear to have resulted from secondary contact following Pleistocene range changes. If so, this would point to a long term stability in the equilibria maintaining the zone. Unlike many plant hybrid zones (Stebbins, 1974), most hybrid zones in animals are not associated with ecotones, which suggests they are maintained by hybrid unfitness, rather than differential ecological adaptations.

The structure and complexity of hybrid zones, and their obvious relevance to species and speciation concepts, are intellectually very seductive and a large body of work has been done by both empirical and theoretical evolutionary biologists. This literature has been reviewed by Barton & Hewitt (1981,1985), Bigelow (1965), Harrison & Rand (1989), Hewitt (1989), and Woodruff (1973,1981). The most comprehensive summary of mathematical models and computer simulations of hybrid zones are to be found in Barton & Hewitt (1985) and the works of Barton cited therein.

Postzygotic developmental incompatibilities appear to be the most important factor limiting gene flow across hybrid zones. If at least one sex is fertile in hybrid zone populations, a great deal of genetic variation will result from backcrossing, the uniting of individuals of mixed genetic background, and the effects of recombination. Indeed, hybrid zone phenotypes may be poorly correlated with other indices (allozymes, fertility, etc.) of hybridity. Many hybrid zones contain "hybrids" with complex genotypes (or this is inferred from overall phenotypic variation) and yet the zones are narrow with respect to estimates of dispersal rates. One might expect natural selection to act on this range of genotypes to increase compatibility and thus lead to a fusion of the interbreeding taxa. The narrow width of some hybrid zones suggests that incompatibilities result from disharmonious gene interaction at many loci such that recombination tends to break up harmonious gene combinations as they arise. Gene flow from outside the zone would have the same effect.

In the absence of a detailed knowledge of the genetics of incompatibilities, most workers have used allozymes and morphological characters as markers to measure zone width and to construct character clines away from the zone. If only a few loci with large effect control hybrid fitness, neutral or advantageous alleles from one interbreeding taxon should introgress across the zone and form long clines into the other population. This will not occur if such alleles are closely linked to any deleterious gene. Thus, if many fitness loci are involved, hybrid zones can form strong barriers to gene exchange, even in the complete absence of premating barriers. This would be especially true for species with low dispersal rates. Barton & Hewitt (1989) and Hewitt (1989,1990) argue that a species could be dramatically subdivided into genetically distinct populations through the action of environmental disturbance (e.g. glaciation) and the consequent formation of parapatric hybrid zones.

Bigelow (1965) points out that when selection favoring improvement of reproductive isolation is strongest, i.e. when hybrid unfitness is highest, then gene flow actually will be lowest into bordering parental populations. The great majority of matings either side of the hybrid zone will be between "pure" genotypes. The hybrid zone itself, through incompatibilities, isolates the two interbreeding forms.

In summary, hybrid zones can reveal much about the genetics of speciation. They provide a laboratory for testing theories about the

nature and origin of premating barriers. Genetic differences between the interbreeding taxa causing disruption of postzygotic development probably represent the kinds of critical genetic changes leading to speciation. Unfortunately, we cannot know the time span between such differentiation and the formation of a given hybrid zone. We must also bear in mind that speciation can occur by the formation of strong premating isolation with little or no postzygotic incompatibility. Closely related species in this class will not likely form hybrid zones. The great deal of genetic variation generated in hybrid zones could potentially lead to novel adaptations. The ability of hybrid zones to block introgression, however, may limit the role this variation can play in evolution.

7. Sympatric speciation.

Models of sympatric speciation describe conditions under which reproductive isolation could theoretically arise on a distance scale comparable to the average dispersal of breeding individuals in a population (Bush, 1969; Diehl and Bush, 1989; Tauber and Tauber, 1989). The model requires a close association between mating and host choice, both behaviorally and genetically. Mating would occur on the host and genes controlling mate choice and host preference would consist of single loci closely linked, thus reducing the effects of recombination. If a new allele arose by mutation which adapted the organism to a new host (by altering its digestive enzymes, for example) selection would tend to favor compatible variation in the gene controlling host association and thus mate choice. Homozygous matings would be favored if heterozygotes were ill-adapted. A new host race or species could arise in this manner. The model has been criticized on the grounds that the genetic system is unrealistically simplified, and that when the new allele first appears matings with parental genotypes would occur and tend to break up gene combinations favoring the host shift (Futuyma and Mayer, 1980; Butlin, 1987). While some organisms, such as the *Rhagoletis* (Diptera:Tephritidae) fruit flies studied by Bush, seem to fit the model, most Lepidoptera in natural environments do not mate in such close association with their hosts and many exhibit some degree of dispersal in response to pheromones or during courtship behavior. Rapid shifts in host choice could accompany speciation in Lepidoptera in the context of range expansion or colonization. It may not be possible to know if change in host plants is a cause or an effect of range expansion. In evaluating the many putative examples of sympatric speciation it is difficult to define criteria which would exclude allopatric models.

Summary

No single process controls the genetic changes leading to reproductive isolation between closely related animal populations. Reproductive isolation may arise incidental to the evolution of the ecological, morphological and physiological traits we use to characterize species. Alternatively,

reproductive isolation may be poorly developed among taxa otherwise rich in taxonomic character differences. Strict application of the isolation criterion of the Biological Species Concept is inappropriate in such cases.

To study speciation we must compare genetic differences between closely related taxa and infer which of these accompanied the origin of reproductive isolation. Experimental hybridization is an important tool in this regard. One of the challenges for the evolutionary biologist is to distinguish the genetic differences among species from the genetics of speciation.

Populations of species may become differentiated through the action of selection, genetic drift and historical events affecting demography; the roles of gene flow and coadapted gene complexes in countering this divergence and maintaining species integrity may be less important than once believed. Under conditions of reduced gene flow, strong selection gradients may produce character clines or otherwise act to differentiate populations. Theoretically, it is impossible to distinguish primary from secondary differentiation. Genetic compatibility may decrease within a species when test crosses are made with individuals from increasingly distant populations. Although taxonomy must deal with discrete species, single populations are the ecological and evolutionary units of change. Speciation does not necessarily require a major restructuring of the genome; many of the fixed differences in allozymes between species are present as polymorphisms within closely related species.

Features which traditionally have been termed "reproductive isolating mechanisms" may actually arise as products of selection favoring increased reproductive fitness for "mate recognition" within a population. Subsequent isolation from related taxa is then a byproduct of this process. Contrary to the predictions of the Biological Species Concept, theoretical arguments and empirical data do not support the routine improvement of isolation between species which have come into secondary sympatry or which have formed hybrid zones. The *Drosophila* may be an exception to this rule.

Populations lacking effective prezygotic isolation may become significantly different for traits affecting postzygotic development in their hybrids. Upon secondary contact such taxa form hybrid zones, the structure of which is largely determined by the opposing effects of hybrid unfitness and gene flow. The study of hybrid zones has been profitable in understanding the genetics of speciation.

PART II. Representative Studies.

I have chosen especially well-documented studies in five taxonomic groups to illustrate the concepts of population differentiation and speciation presented in Part I. These genera or species groups share at least some aspect of natural hybridization, and by this criterion deal with taxa near the species boundary of evolution. All the studies involve extensive

field and laboratory research using various methodologies, as contrasted to taxonomy based solely on comparative morphology. An ideal program for studying speciation might use experimental hybridization to understand pre- and postzygotic genetic compatibility, and morphometrics and biochemical genetics as independent measures of genetic similarity and gene exchange between populations. No single example here employs all these approaches. In particular no direct observation of mating behavior or prezygotic isolation was made for any group except the *Hyalophora*, where the pheromone mating system is more easily manipulated than the complex courtship of butterflies.

For the purpose of stimulating thought and discussion, I offer in some cases alternative interpretation of likely modes of population differentiation and speciation. Thus, for the tiger swallowtails, I give evidence for a secondary contact between full species in place of an ecological model of primary intergradation between *glaucus* and *canadensis*. Similarly, for the *Limenitis* problem I propose for consideration a secondary intergradation/hybridization model for the blend zone between *arthemis* and *astyanax*, although in this example definitive allozyme and experimental hybridization data are not yet available. The clines in mimetic morphs in *Heliconius* illustrate how selection can maintain a dramatic shift in phenotype over a short geographic distance. Yet the genetic differences between these subspecies appear to be relatively minimal. In the *Hyalophora* premating barriers are lacking and the degree of postzygotic isolation in hybrids among the various taxa reveals a hierarchy of levels of speciation.

The Tiger Swallowtail Species Group.

These large, attractive butterflies are well known to collectors yet still pose new and interesting questions concerning species relationships. The eastern *Papilio glaucus* has a female polymorphism: a yellow form differing slightly from the male, and a dark morph believed to be a mimic of the distasteful *Battus philenor*. The three western species are *eurymedon*, *multicaudatus*, and *rutulus* (which most closely resembles *glaucus*). No dark female morph occurs in the western species.

Clarke & Sheppard (1955, 1957, 1962) hybridized *glaucus* with *rutulus* and *eurymedon* to study the genetics of the dark morph. Their results indicated significant genetic differences between *glaucus* and the western forms. In *glaucus* the locus controlling the dark morph appeared to reside on the Y chromosome, but the expression of this gene was blocked in hybrids with the western species. Prolonged or "permanent" diapause in female pupae may occur commonly in these hybrid crosses, although this may be overcome by injection of ecdysone (Clarke & Willig, 1977). A similar diapause disruption occurs with *glaucus* × *multicaudatus* crosses (West & Clarke, 1988). These crosses were made using the technique of hand pairing; nothing is known concerning premating barriers in nature.

Brower (1959ab) investigated species relationships within this group

by means of morphology, and field observations. He attempted to establish the existence of hybridization between *glaucus* and *rutulus* using a single wing character and slight differences in male genitalia. Areas of suspected hybridization occurred in British Columbia and the Black Hills of South Dakota. On the basis of genitalia alone certain specimens intermediate for wing spot color in each locality would have been classified as *glaucus*. Brower (1959a) noted that the most convincing data for natural hybridization are for the Black Hills, where *rutulus* is rare compared to *glaucus*.

To date these populations have not been reexamined using modern methods of multivariate analysis or surveys of allozyme variation. No series of putative hybrids have been illustrated in any publication. Yet, recent literature treats the existence of hybrid zones as well established. Scott (1986) reduces *rutulus* to a subspecies of *glaucus*, presumably on the basis of putative hybrid zones, but without a discussion of justifying criteria.

Scriber (1983,1984) has extensively documented geographic variation in host plant adaptation in the *glaucus* group and the genetics of sexual dimorphism in *glaucus* (Scriber et al., 1987). The experimental basis for this work has been hybridization with a northern taxon, *canadensis*, considered a subspecies of *glaucus*, which lacks the dark female morph, is univoltine throughout its range, is smaller than *glaucus* and differs in several wing pattern characters. Hybridization and controlled rearing experiments have shown the following: 1) the gene for dark morph in *glaucus* is carried on the Y (W) chromosome and is suppressed by an X-(Z-) linked locus in *canadensis*; 2) obligate diapause in *canadensis* is controlled by a sex-linked locus, while *glaucus* has a facultative diapause responsive to daylength and controlled by autosomal loci (Hagen & Scriber, 1989; Rockey, et al., 1987ab); 3) hybrid female pupae typically enter a prolonged or indefinite diapause, as in the interspecific crosses described above. While both forms feed on Wild Black Cherry, they are each unable to metabolize a common host of the other taxon - Tulip Tree for *glaucus* and Quaking Aspen for *canadensis*. Hybrid F₁ survive on all three hosts.

The two taxa are parapatric in the Great Lakes region and in the Northeast. Obviously, a clear understanding of the origin of ecological and physiological differences between *glaucus* and *canadensis*, and moreover the nature of species and speciation in this group, rests on a correct interpretation of contact zones between the various taxa.

Scriber et al.(1987), Scriber & Evans (1988), and Luebke et al.(1988) describe the interaction in Wisconsin as a "hybrid zone" between subspecies. Scriber (1983) proposes that ecological factors determine the transition zone between subspecies. The northern limit of *glaucus* corresponds to the demarcation of 1200 - 1300 degree-days, which lab studies show to be the northern limit for completion of a second brood. This region fairly closely parallels the southern extent of Quaking Aspen and thus

the southern range limit of the *canadensis* phenotype. Importantly, all diagnostic ecological, morphological, and physiological characters change concordantly and abruptly in a north-south transect. There is no evidence for clines in polymorphism in host plant adaptation, dark morph frequency, or voltinism as one would expect for overlapping, interbreeding subspecies.

The only evidence for interbreeding in the Great Lakes region comes from morphometric analysis using lab hybrids as a third reference group. This analysis was made difficult by variability in key characters in the lab hybrids (Collins & Luebke, unpubl.; Luebke, 1985; Luebke et al., 1988). Only two wing pattern variables plus wing length are statistically significant, resulting in relatively large misclassification errors: 19.3% for reference lab hybrids and 8.3% for *glaucus* reference specimens (incorrectly classified as hybrids). The results for the transition zone in Dane Co. were 2.7 % *canadensis*, 12.2% hybrids, and 85.1% *glaucus* for a sample of 74. It is unclear if these phenotype ratios can be found in a single local population, but if so the presence of parental phenotypes indicates either assortative mating or selection against hybrids.

It is possible that both the hybrids and *canadensis* scores are at least partly due to misclassification of *glaucus*, as noted above. Another possible source of error is confusion arising from the resemblance between the "spring form" of *glaucus* with *canadensis*. Ideally, the *glaucus* reference group should be composed only of these spring adults.

The Wisconsin transition zone thus appears to be a case of parapatry between morphologically, ecologically, and physiologically distinct species. They are partly isolated by postzygotic incompatibility in diapause physiology, and the morphometric evidence for interbreeding is inconclusive. The concordant and abrupt discontinuity for key distinguishing traits is highly suggestive of either very limited hybridization with selection against hybrids, or for a narrow zone of sympatry without interbreeding.

Hagen (1990) reports on the contact zone between *glaucus* and *canadensis* in the Northeast, using allozyme variation, host plant suitability, voltinism, and a diagnostic wing character. The two taxa differ in only two enzymes of 13 polymorphic loci tested. Populations were polymorphic at these two loci in three sites in northern Pennsylvania and south-central New York, whereas populations north and south of this region were fixed for alternate alleles. The loci are sex-linked. This region also corresponds with a within-brood intermediate ability to survive on some hosts of both subspecies, and with intermediacy in a wing band trait. Like *canadensis*, "hybrid zone" populations lack the dark female morph and are univoltine. Hagen interprets the contact zone as a very narrow hybrid zone maintained by hybrid unfitness, perhaps due to disrupted diapause in hybrids and/or metabolic "costs" associated with maintaining detoxification systems for the hosts of both taxa. Since the allozyme loci are sex-linked, their sharp frequency clines in the hybrid

zone may be due to close linkage with the locus controlling diapause, rather than to selection directly on the enzyme loci. Hagen ascribes variability in host plant utilization and allozyme frequencies to gene introgression. However, the "spring form" of *glaucus* in New York is known to feed on Tulip Tree yet may resemble *canadensis* in wing markings, lacks the dark morph, and is univoltine at its northern limit (Hagen, 1990; Luebke et al.; 1988; Shapiro, 1974). In two of the three "hybrid zone" locations the allozyme frequencies for one locus closely approximate those of *glaucus* to the south. The best evidence for interbreeding is the very sharp cline in allozyme frequencies for two loci.

The data at present do not allow an estimate of the relative frequency of primary hybrids or the extent of backcrossing and recombination, if any. It is possible that a past period of interbreeding produced the pattern of allozyme variation through introgression into a predominately *glaucus* genome. Climatic variation during the Pleistocene could have resulted in cycles of allopatry between what we now call *canadensis* and *glaucus* followed by range expansion and parapatry. If interbreeding is ongoing, the narrow width of the apparent hybrid zone is more likely due to strong selection on some locus, probably sex-linked, than to the differentiating effects of broader ecological selection gradients.

Very little is known about prezygotic isolating mechanisms in nature. The small female *canadensis* is reportedly difficult to hand mate to larger male *glaucus*, and the flight characteristics of the two taxa are apparently different in terms of plant community association (Scriber et al., 1987; Scriber, pers. comm.) although R. Lederhouse minimizes these differences (pers. comm.). In Wisconsin recent agricultural disruption may have created a patchy plant community association which could promote either limited hybridization or an increase in sympatry between the two taxa in terms of islands of Aspen interdigitating with open fields and fence rows with Wild Black Cherry supporting *glaucus* populations. More extensive morphometric analysis using the spring form of *glaucus* needs to be done in both localities and on a finer demographic scale. Further analysis of these zones using mitochondrial genetics (Hagen, 1990) and multivariate phenetics will help determine the true extent of gene exchange.

Scriber has attributed the occasional occurrence of gynandromorphs and color mosaics in female *glaucus* to a disruption of development resulting from long distance introgression from the putative northern "hybrid zone" into more southern *glaucus* populations (Scriber et al., 1987). This interpretation has been cited and accepted by West & Clarke (1988) in their investigation of the inheritance of the black morph. However, long distance introgression of a deleterious allele is not credible in terms of our current knowledge of hybrid zones. Gynandromorphs and color mosaics have been collected far from the range of *canadensis* and occur in both pure and interspecific lab broods.

The true taxonomic status of *canadensis* must also be considered in future investigation of the western contacts between *rutulus* and the eastern *glaucus* forms. It is interesting to note that *canadensis* resembles *rutulus* in terms of sharing Quaking Aspen as a host, and in the form of the yellow submarginal forewing band and other adult wing characters. Recently, Hagen and Scriber (1991) published an allozyme survey of taxonomic relationships among the tiger swallowtail and *P. troilus* species groups. Genetic relationships inferred from these enzyme data confirm the full species status of *canadensis*, and show a very close relationship between *rutulus* and *eurymedon*. Apparently, in the latter two species rates of divergence have been greater for morphology and ecological characters than for metabolic enzymes; there is no evidence for intergradation in nature.

The Western *Papilio machaon* group of Black Swallowtails.

This relatively small group of butterflies illustrate many of the taxonomic problems one encounters in applying species concepts to morphologically distinct populations which lack effective reproductive barriers (Sperling, 1990). The various taxa exhibit seasonal "forms" (polyphenism), "dark" and "yellow" polymorphism, and discordant clinal variation. In some areas where several taxa are sympatric the various taxa have overlapping morphological variation and no one wing nor genitalic character can reliably separate the various entities.

Sperling (1987) employed a multidisciplinary approach involving lab broods reared from specific hosts, allozyme analysis, and morphometric analysis of 11 key wing and body characters. Multivariate analysis of allozymes, morphological characters, and the combined data set produced similar groupings of individuals and populations, but in some cases adding host plant association as a variable improved species separation. Host plant adaptations appear to play a central role in the evolution of the *machaon* group.

Sperling synonymized with *machaon* the taxa *oregonius* and *bairdii* and all other forms feeding on *Artemisia dracunculus*. *Papilio zelicaon* and *polyxenes* remain distinct species. [*Papilio indra* has a distinct adult morphology, genitalia, and larval phenotype and is not included in this study. This species appears to be reproductively isolated from others in the *machaon* group.] Hybrid zones occur between *zelicaon* and *machaon* (primarily in Alberta), and between *machaon* and *polyxenes* (primarily in Manitoba). Interestingly, these pairs of taxa remain distinct in sympatry in many areas but hybridize to varying extent in others. Hybridization suggested by morphometric analysis was confirmed by comparing allozyme frequencies and calculating departure from Hardy-Weinburg expectations based on free gene exchange. No corroborative laboratory hybridization was done to measure pre- and postzygotic isolation among populations or taxa.

Degree of hybridization seems to depend on the topography and host plant association in the area in question, probably in terms of the effect on mating behavior, especially "hilltopping". In the Riding Mts. of Manitoba habitat disturbance by man may have promoted recent hybridization, but other hybrid zones probably arose during the Pleistocene. In nature it appears that *machaon*, *zelicaon*, and *polyxenes* are isolated by ecological characteristics more than by mating barriers or postzygotic incompatibilities. Experimental hybridization (Clarke et al., 1977; Remington, 1968a) indicates minimal postzygotic genetic incompatibility in terms of fertility, embryo viability, disruption of diapause, or adult sex ratios. These findings are in contrast to the Tiger Swallowtail group discussed above. Nevertheless, more extensive hybridization should be done, especially comparing populations in hybrid zones with areas of successful sympatry.

Sperling (1987, 1990) believes that speciation and differentiation within species in the *machaon* group occurred in allopatry in various refugia during the Pleistocene. The Umbellifer feeding ancestors of *machaon* apparently shifted to *Artemisia dracunculus* and became subdivided into various subspecies. The present distribution of clines, hybrid zones, and regions of successful sympatry are probably the result of range expansion within the last 10,000 or so years.

The studies of Thompson (1988ab) on the genetics of oviposition preference and specialization complement the work of Sperling. Female *zelicaon* and *machaon oregonius*, bred from wild-collected iso-female strains, were allowed to oviposit in cages on *Cymopterus terebinthinus*, *Lomatium grayi* (both native Umbellifer hosts of *zelicaon*), *Foeniculum* (an introduced Umbellifer host of *zelicaon*), and *Artemisia dracunculus* (the normal *machaon* host). Both species laid preferentially on their native hosts and the ranking order was consistent overall within each species. Yet, within each species, the iso-female strains did differ in degree to which females laid some ova on hosts of the other species. Within strains of *m. oregonius* some females laid ova on all four hosts, while others laid only on the natural *Artemisia* host. Within strains of *zelicaon* females differed in the ranking of their normal hosts, but females within all strains laid at least some ova on *A. dracunculus* and significantly more on *Foeniculum*.

Thompson interprets the variation in oviposition preference among strains and within strains as evidence of genetic variation controlling this behavior. Thus, these butterflies seem to have speciated by means of the genetic-based flexibility to undergo a host shift, based on oviposition behavior. Under novel selection favoring a host shift, as during range expansion into new plant communities, the genetic potential exists in latent form rather than requiring mutation. Future research may reveal more detail about the underlying genetic basis and variation among populations with respect to host plant adaptations.

Intergradation between *Limenitis arthemis* and *L. astyanax*: hybrid zone or selection along a cline?

The zone of phenotypic intergradation between the white-banded *Limenitis arthemis* and the unbanded form *L. astyanax* was the subject of a now-classic study by Platt and Brower (1968). They interpret the zone of intergradation as an area of relaxed selection between the northern banded form, which benefits from a wing pattern disrupting the outline of the adult, and the dark blue unbanded form, which is seen as a Batesian mimic of the unpalatable *Battus philenor*. In their model no other restrictions on gene exchange exist, the two forms are considered conspecific, and the distribution of phenotypes represents an example of adaptive response to a gradient of selection, in other words primary intergradation in the sense of Endler (1977). The opposing view is that the two forms are actually well-differentiated and are hybridizing as a result of secondary contact (Remington, 1968b, 1985). The present data do not allow us to resolve this controversy, but the problem is worth reviewing in some detail because it embodies so many key questions in speciation theory.

The southern limit of the disruptive pattern phenotype is approximately concordant with the southern limit of Ice Age glaciation and so the present distribution must represent recolonization. Old World and western North American *Limenitis* are banded and this is assumed to be the ancestral phenotype pattern. The zone of intergradation is about 160 km wide. Correspondingly, the northern extent of the unbanded phenotype is roughly the same as the northern limit of the mimicry model, *Battus philenor*.

A selective basis for the mimetic *astyanax* phenotype has been established experimentally by demonstrating that *B. philenor* is unpalatable and that birds avoid the *astyanax* phenotype after exposure to *philenor* (Brower, J.V.Z., 1958; Brower & Brower, 1962; Platt et al., 1971). The supposedly disruptive value of the *arthemis* phenotype has not been tested, which would probably require difficult field experiments, since a flight behavior component may complement the wing patterns. No experiments have been performed on the probability of predation on phenotypically intermediate phenotypes.

Platt and Brower (1968) and Platt (1983) reason that since the two forms are conspecific then the zone of intergradation must be of primary origin, and not a hybrid zone of secondary contact. They cite three categories of evidence for conspecific status: 1) wild-caught and reared material show no deviation from a 1:1 sex ratio nor departure from expected phenotype frequencies which might occur due to hybrid postzygotic incompatibility (but see below); 2) the two phenotypes share identical genitalic structures; 3) hybrid broods showed no gross evidence for developmental incompatibility.

Several points of criticism can be made, especially of the 1968 study, in light of recent advances in concepts and methods of investigation. Most

of the data on sex and phenotype ratios in Platt & Brower (op. cit.) are based on crosses made with the same male individual and most females are from one mating. No replicate crosses have since been published. Quantitative studies of fertility, embryo viability, diapause disruption, emergence schedules, and hybrid female fecundity should be done, comparing different phenotypic classes and geographically separated populations, as in the cited work by Oliver. Tests of the genetic model of wing pattern inheritance proposed by Platt (1983) might provide an estimate of the number of loci controlling the expression of the mimetic phenotype. Platt (1975) and Platt et al. (1978) have investigated the wing pattern genetics of the monarch mimic, *L. archippus*, as well as the genetic compatibility of this species with *arthemis/astyanax*. Finally, as noted in the review of concepts above, genitalic structure is not necessarily a reliable index of relationships near the species boundary.

The Hardy-Weinberg analysis of phenotype ratios (Platt & Brower, op. cit.) is flawed because it rests on pooling data from many subpopulations or demes rather than one large interbreeding population. This results in a tendency to record a lower number of heterozygotes than exist in the population. This distortion can also result from observation of the same population over long time periods. As noted by Platt (1983) this type of criticism is inherent in the limitations of the Hardy-Weinberg test, and is referred to as the Wahlund Effect (Hedrick, 1983, p. 284; Lewontin & Cockerham, 1959). If one could determine the boundaries of an interbreeding population, the Hardy-Weinberg test for selection would be more robust. However, the relative deficiency of intermediate phenotypes (presumed heterozygotes) attributed to the Wahlund Effect by Platt could also be ascribed to selection. The Hardy-Weinberg analysis as a test for selection is compromised further by likely violation of one or more of its assumptions: no gene flow, no effect from genetic drift, no mutation, and the requirement for random mating. With regard to natural selection, there is a self-contradiction in arguing for conspecificity from a Hardy-Weinberg analysis in this case, since agreement with predicted frequencies rests on the assumption of no selection, yet differential selection on wing morphs is what the authors are attempting to prove. But to prove conspecificity Platt and Brower (op. cit.) try to establish that the frequency of heterozygotes (inferred indirectly from phenotype) does not differ from an expected value based on absence of selection against hybrids, i.e. no postzygotic isolation. The contention that the 160km wide overlap zone is an area with "no selection" on wing phenotype is unlikely on general principles (Endler, 1986, espec. ch.4) and unproven from the data. To postulate a condition of no selection in this case requires that each phenotype class has the same survival potential in the face of predation and all other agents of selection.

The Hardy-Weinberg test suffers from an insensitivity to certain types of selection including reduced fecundity and sexual selection (Endler, op. cit. p.65), either of which might be operating in nature in this case. A

further complication arises from estimating heterozygote frequencies from intermediate phenotypes, since a range of intermediate morphs occur and modifier loci are postulated to operate in the expression of the mimetic *astyanax* phenotype. Hardy-Weinberg in this application is based on a simple one-locus Mendelian inheritance.

In contrast to Platt and Brower's taxonomic arguments, the two forms can be conspecific and the intergradation could also have arisen by secondary contact. The two conditions are not mutually exclusive. There seems to be no supporting evidence that an allopatric model is "unnecessarily complex" (Platt & Brower, op. cit.) compared to a model of primary differentiation along a gradient of variable selection. Furthermore, the selection regime (agent of selection, environment, population structure, etc.) present during the evolution of wing pattern differences almost certainly differs from the present situation. Pleistocene range changes could have separated the *Limenitis* into allopatric populations. The mimetic form could have evolved as an isolated population under conditions of more intense selection than are experienced now on the average.

Indeed, a wide zone of intergradation would in itself suggest very weak selection on mimetic versus disruptive wing patterns, which makes a primary intergradation model less convincing. Although we know little about dispersal rates in these butterflies, selection during the evolution of the mimetic pattern in the face of gene flow would necessarily have to be relatively strong to produce the genetic changes regulating the coordinated expression of various pattern elements. It is also possible that habitat alteration by man has increased the width of the blend zone.

In fact, the width of the intergrade zone in *Limenitis* is considerably greater than average compared to those hybrid zones listed by Barton & Hewitt (1985), which would tend to support the concept of minimal genetic incompatibility in hybrids between *arthemis* and *astyanax*, in agreement with the Platt and Brower model and in contradiction to the two species - hybrid zone model of Remington (op. cit.). However, the presence of intermediate and both "parental" phenotypes in local populations presents a difficulty. Random mating with no differential selection on adult phenotypes should break down the genetic basis for parental phenotypes through recombination, especially if modifier loci are involved in the expression of the *astyanax* phenotype. The persistence of both the *astyanax* and *arthemis* phenotypes suggests a highly "canalized" development such that expression of the wing pattern is stable over a range of genotypes, and/or that selection has an effect on phenotype frequencies.

Historical range changes must also be considered in understanding the present blend zone. It is important to note that the narrow-banded *arthemis*-like form "albofasciata" occurs quite far south well into the range of *B. philenor*, and even south of the zone of hybridization (Clark & Clark, 1951; Platt, 1983; Shapiro, 1966; Shapiro, pers. comm.). The persistence of the northern *arthemis* phenotype in these southern loca-

tions may represent the present effect of relict populations left behind as changing climate in the Holocene allowed the *Limenitis*, and their zone of intergradation, to move northward. Interestingly, a more abrupt cline in *arthemis/astyanax* phenotype frequencies was found by Waldbauer et al. (1988) in the Upper Peninsula of Michigan, but lake barrier effect complicates interpretation of the relative importance of gene flow and selection.

An alternative interpretation is that the blend zone represents range overlap where genotypes producing both the *arthemis* and *astyanax* phenotypes are adaptively superior to intermediate forms, but weak mating barriers result in continuing hybridization between parental forms. Some degree of genetic incompatibility appears to limit the genetic recombination resulting from backcrossing and therefore hybrid-like phenotypes are present in a lower frequency than in hybrid zones such as the *Hyalophora* in the Sierra Nevada (discussed below). The unusually wide zone in this model is due to range overlap with limited hybridization, in contrast to more typical narrow hybrid zones composed of intermediate and recombinant phenotypes bounded by clines into parental populations. As noted above, the unusual width of the zone may also be due to historical range changes. This interpretation is similar to that of Remington (1968b, 1985) in that it hypothesizes secondary contact producing hybridization in a zone of range overlap. Unlike the Remington model, I do not postulate the subsequent origin of reproductive isolation. These *Limenitis* appear to be more genetically distinct than alternate morphs in a polymorphism, but less divergent than full species.

Allozyme analysis would provide an independent measure of genetic similarity between *arthemis* and *astyanax* and could detect the true extent of overall gene exchange across the intergrade zone. Electrophoresis can provide unambiguous identification of heterozygotes, and if significant or fixed allele frequency differences occur between pure *arthemis* and *astyanax* reference populations, then the population genetics of the zone of intergradation might be better understood. Finally, allozyme analysis could produce a truer index of hybridity for phenotypically intermediate specimens. As noted in the hybrid zone discussion in Part I, hybrid zone phenotypes often do not accurately reflect underlying genotypes.

Evolution in these *Limenitis* stands in contrast to the *Papilio glaucus* / *canadensis* situation in which *glaucus* seems to have evolved a mimetic form, also based on the *Battus philenor* model, yet appears to be reproductively isolated from the very similar, non-mimetic *canadensis* to the north. The respective ranges of the two pairs are similar as *arthemis* is nearly concordant with *canadensis* and *astyanax* is approximately sympatric with *glaucus*. These shared distribution patterns in unrelated groups are evidence of a common response to changing climates during and after the Ice Age. Remington (1968b) hypothesizes that such concordant "suture zones" formed as biota rejoined following a period of

allopatric divergence as relict populations, in this case in refugia in the southeastern United States.

Selection along a cline between alternate mimetic morphs in *Heliconius*.

Heliconius erato and *H. melpomene* are brightly colored, unpalatable tropical butterflies which occur sympatrically as Mullerian mimics. By closely resembling each other, each species benefits because their combined numbers in a population increase the rate at which predators learn to avoid attacking any butterfly with the distinctive warning color phenotype. By contrast, a Batesian mimic, which by definition is palatable, cannot theoretically exist in a population at a frequency higher than its distasteful model. These *Heliconius* are especially interesting because they exhibit parallel polymorphisms. Distinctive color morphs in different populations of the more common *H. erato* are often accompanied by similar geographic variation in the wing patterns of *H. melpomene*. Mallet and Barton (1989ab) and Mallet et al. (1990) have extensively studied the nature of selection on these shared polymorphisms in regions of Peru where wing phenotype clines connect populations of distinctive, alternate warning color patterns.

The population genetics of these clines represent a special case of "frequency dependent selection" wherein for a given population the most common phenotype (of either species) has a selective advantage over the alternate, less frequent morph. This is true because predators are more likely to encounter the more common morph. Since a certain minimum number of encounters is needed for a "naive" predator to learn to avoid a warning color pattern, the less common of two alternative morphs will suffer disproportionate predation. Thus, given regions support populations of the two *Heliconius* which have very similar morphs at high frequencies. Adjacent regions may have populations where the alternate morph is present at a high frequency in both species. The two areas are connected by very abrupt clines in wing morph type.

Mallet and Barton (1989a) estimated the strength of selection maintaining these clines using marked foreign (experimental) and native (control) butterflies which were released and subsequently recaptured at intervals along clines between regions supporting alternate wing morph populations. Their analysis showed that losses among odd morph experimental individuals occurred soon after release, most probably due to predation, since released butterflies did not disperse and tended to join communal roosts irrespective of wing phenotype. Within each species, populations with differing wing patterns showed no significant differences in allozymes, nor any important ecological distinctions. Alternate wing morphs are controlled by three loci in *H. erato* and by four loci in *H. melpomene*. The inference is that cline structure in each species is a function of selection on these loci, rather than due to more complex hybrid incompatibility or differing ecological adaptations. Estimates of the

coefficient of selection in *H. erato* was quite high, about .17 per locus, or about a 52% selection against experimental individuals released into populations of the opposite warning color phenotype.

Because the genetics of this polymorphism are well understood, Mallet and Barton (1989b) and Mallet et al (1990) were able to compare their experimental data with simulation models of selection along clines. From experimental studies they obtained allele frequencies for wing pattern loci along clines between populations with differing wing morphs. In their models, cline width can be expressed as a function of gene flow and selection. Gene flow also causes a linkage correlation (linkage disequilibria) between loci that differ along a cline. Thus, they used observed cline width and linkage relationships to estimate gene flow and selection. Estimates for selection generally agreed with those obtained from mark and recapture studies. The usefulness of various computer simulations of clines was confirmed. Moreover, these analyses tended to confirm theoretical models of the evolution of Mullerian mimicry based on a few loci with large effect.

Mallet and Barton investigated the *maintenance* of a cline between genetically differentiated populations without directly addressing the *origin* of these genetic differences. The approach of Platt and Brower to the *Limenitis astyanax/arthemis* problem explicitly equated origin and maintenance in terms of primary intergradation due to differential predation on wing pattern morphs. However, the occurrence of parental phenotypes at high frequency in the broad *Limenitis* blend zone suggests that these forms are differentiated beyond the level of polymorphic conspecifics.

The natural experimental system represented by the mimetic morph clines in *Heliconius* was more amenable to study than the situation in the *Limenitis* because 1) the genetics of the polymorphism were understood; 2) the inference of selection based on predation was not subject to uncertainty concerning the possible added effect of hybrid unfitness; 3) allozyme data established that the taxa are conspecific; 4) selection was measured directly by field experiment rather than by statistical inference from Hardy-Weinberg calculations; and 5) the width of the zone was narrow enough that mark-recapture techniques could be used simultaneously across the entire zone. The narrow width was also partly due to the strength of selection, which in turn aided the interpretation of the mark-recapture data.

Natural Hybridization in the genus *Hyalophora*.

The members of this genus of large, attractive saturniid moths readily hybridize in captivity, generally producing fully viable adults. Female hybrids are usually barren, but males can be backcrossed and even three or four nominate taxa have been combined in the genomes of lab hybrids. Natural hybridization occurs to varying degrees in areas of congener sympatry. By the Recognition Species Concept this genus would contain

only a single species, although this viewpoint would ignore important morphological, ecological, and genetic differences.

Sweadner (1937) was the first to study natural hybridization in *Hyalophora* and his work was an early and important recognition that traditional morphological criteria are inadequate in describing species boundaries for groups such as the *Hyalophora*. We now know that this genus contains a hierarchy of taxa as judged by the degree of reproductive isolation among the various taxa. Tuttle (1985) showed through a series of careful field tests in Michigan that the large, eastern *H. cecropia* is partially isolated by seasonal and diurnal separation in flight activity from interbreeding with the smaller, dark *H. columbia*. Occasional hybrids occur in nature (Collins, 1973; Ferge, 1983; Sweadner, op. cit.) but no true hybrid zone occurs between these taxa.

By contrast, a zone of intergradation in Manitoba and Ontario connects populations of *columbia* with the larger, brighter colored *H. gloveri* which occurs in the Canadian Prairie Provinces and south through the Rocky Mts. and Great Basin. Both adult and larval phenotypes intergrade and blend zone females oviposit on hosts of *gloveri* in addition to the conifer *Larix* (tamarack), on which the eastern *columbia* is a specialist (Collins, op. cit.; Kohalmi & Moens, 1975, 1988). Laboratory hybrid females between these taxa are typically fecund, in contrast to crosses between *cecropia* and other congeners (Collins, op. cit.; unpub. data). Lemaire (1978) synonymizes *gloveri* as a subspecies under *columbia*.

Hyalophora euryalus on the west coast is quite distinct from *gloveri* in all stages, yet the two species form a large hybrid zone on the east slope of the Sierra Nevada south of Lake Tahoe in California (Collins, 1984). Multivariate analysis shows that adult phenotypic variability in mid hybrid zone greatly exceeds that seen in lab reference F_1 hybrids; extensive backcrossing and recombination appear to be responsible for this variation, not merely the production of primary hybrids each season. While crosses between widely separated population of *euryalus* and *gloveri* produce barren female hybrids, females with intermediate or recombinant phenotypes from the hybrid zone are fully fecund. Moreover, in test crosses using females derived from various sites along a transect across the hybrid zone, genetic compatibility was optimal with males from the source population, but decreased with males from more distant populations, even with as little 15km separation. Collins (1984, ms in prep.) interprets this result as evidence for the regional elimination through selection of incompatible genotypes. Local optimization may be aided by restrictions on gene flow due to mountainous topography, although these moths are known to be quite vagile. Genetic compatibility data on the fine structure of hybrid zones are rare, since most subject organisms are not as easily experimentally hybridized. In spite of obvious morphological differences between *euryalus* and *gloveri* in all stages, relatively few loci controlling gametogenesis may regulate the genetic structure of this hybrid zone. It would be important to verify this

model by means of allozyme or other biochemical genetic test of genetic differentiation.

Sweadner (1937) attempted to document by use of a hybrid index the existence of an intergrade zone in northern Idaho and western Montana between *gloveri* on the east and *euryalus* on the west. Ferguson (1972) treats this population, referred to as "*kasloensis*", as a melanic northern subspecies of *euryalus*, based primarily on genitalic structure. A recent reanalysis (Collins, ms in prep.) has revealed that "*kasloensis*" is intermediate and hybrid-like for several wing pattern characters, resembles *gloveri* in early larval stages, yet possesses a unique mature larva phenotype. Morewood (1991) illustrates a similar phenotype for British Columbia "*kasloensis*". The unusual red dorsal scoli pigmentation of "*kasloensis*" may be the expression in a hybrid genome of a gene in the fifth instar which is normally "turned on" only in the penultimate instar of *gloveri*. The dorsal scoli of *euryalus* are yellow in both the 4th and 5th instars. The cocoon resembles that of a lab *gloveri* × *euryalus* hybrid. Females from the intergrade zone are fully fertile, and have a decreased compatibility when crossed with *gloveri* compared to near-normal fertility and viability in hybrids with *euryalus*. Judged by several criteria, the "*kasloensis*" intergrade population seems to be of hybrid origin, but appears to be restricted in gene exchange with *gloveri* to the east, probably due to decreased host plant availability as a result of a rain shadow effect of the Bitterroot Mts. along the Idaho-Montana border. There is an abrupt transition to the *gloveri* phenotype to the east and a more gradual intergradation into *euryalus* in British Columbia. Nevertheless, the "*kasloensis*" population maintains genetic integrity from the swamping effects of gene flow from *euryalus*. It is unknown at present if this equilibrium is due to intrinsic genetic compatibility factors or the effect of ecological selection.

Two other hybrid populations occur between *euryalus* and *gloveri* in Idaho, each much different from the "*kasloensis*" population (Collins, unpubl. data). A hybrid swarm of great phenotypic variability occurs northeast of Boise in Clear Creek Canyon. The adults more resemble the range of phenotypes seen in the Sierra Nevada hybrid zone, which may be due to a more balanced gene input from the two parental populations, *euryalus* from the northern, panhandle region of Idaho, and *gloveri* from near the Sun Valley/Ketchum area.

An extension of Great Basin habitat occurs in southeast Idaho and is occupied by nominate *gloveri* which extends north through the Salmon area to Lost Trails Pass, where the Bitterroots and the Continental Divide merge. This pass appears too high to support a continuous population of *Hyalophora*, but an occasional hybrid-like individual can be taken in the *gloveri* population just to the south and an occasional *gloveri*-like moth occurs in the "*kasloensis*" population just north of the pass. Either limited dispersal occurs over the pass or a period of more extensive gene exchange occurred during a warmer interglacial period.

The hybrid *Hyalophora* populations in the northwest illustrate the fact that unique population genetic factors acting in each situation have produced three very different hybrid zones, regardless of the fact that the same two species are interbreeding in each case. Topography and plant community distribution, and historical climatic changes no doubt played a role in shaping the present structure of these hybrid zones. By extension, all the *Hyalophora* hybrid populations discussed here verify the general premise that individual populations are the true units of ecological and evolutionary change.

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NOTE ADDED IN PROOF: In a recent paper, Hagen et al. (1991) corroborate the full species status of *P. canadensis* using physiological and genetic data. Allozyme data indicate limited hybridization across a very narrow zone in Michigan.

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