

Chapter 5: Speciation Mechanisms and Behavior

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Species are groups of actually or potentially interbreeding populations, which are reproductively isolated from other such groups.

Mayr 1942

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Biological Isolating Mechanisms

Ernst Mayr's (1942) formulation of the **biological species concept** clarified a vexing question for evolutionary biologists: What is a species? Mayr's view of the species concept has widespread acceptance owing to its simplicity. Nevertheless, the manner in which species arise by the process of natural selection remains controversial. The problem is that the process of speciation takes a very long time to complete, and it occurs on a very large geographic scale. In addition to Mayr's definition additional genetic concepts are also useful in delimiting species.

Hybrid sterility definitely separates two species, although it is not the sole criterion. Hybrid sterility is a special instance of **reproductive isolation**. In cases of reproductive isolation between species the barriers to mating do not just have to be due to incompatibility between genomes. The blocks could arise at various levels. These **ecological, behavioral, physiological, morphological or genetic isolating mechanisms**, which serve to limit gene flow between species, are categorized in terms of pre- vs. post-mating mechanisms (Endler, 1977):

A) Premating isolating mechanisms -- prevent union of gametes that produce a zygote

1. mates do not meet (seasonal or habitat isolation)
2. mates meet but do not mate (ethological or behavioral isolation)
3. mates meet but no sperm transfer occurs (mechanical isolation)

B) Post-mating isolating mechanisms -- varying degrees of hybrid sterility or fitness (which are largely due to gene interactions).

1. sperm transferred but dies before fertilization
2. zygote dies during development
3. zygote produces an F1 adult that has reduced viability (survival)
4. hybrid is viable, but it is partially or completely sterile (fecundity) or the F2 is in some way deficient.

Asking the following questions reveals the answer to the adaptive value of pre- versus post-mating isolating mechanisms. Which of the two mating isolation mechanisms is the most effective at reducing the risk of producing low fitness offspring? Which mechanisms are most efficient in terms of time and lost reproductive opportunity?

An organism that mates with a different "semispecies" may produce low viability offspring and this individual would have lower fitness compared to an organism that discriminates against such semispecies and mates with members of its own semispecies. Evolving species discrimination mechanisms is clearly an advantage over one that only possess post-mating isolating mechanisms. Such behavioral discrimination ability should evolve rapidly in the area of incipient speciation, under the right conditions. For these reasons, the study of behavior is central to understanding the processes of speciation.

An example of premating isolation is found in the flour beetle, *Tribolium confusum*). When a researcher pairs a female from Nigeria with a male from a different locality (e.g., allopatric population from Croatia), viable offspring result. The same is true for the reciprocal cross. However, if a researcher simultaneously pairs the female beetle with a male from the same population and an allopatric population, the female preferentially sires progeny by the same-locality male. The mean relative fitness of the sympatric male, 0.425, is much higher than the allopatric male (0.085). Behavioral observations indicate that the female is less likely to become quiescent during mating attempts by allopatric males than when she is approached by males from her population.

Biological barriers to interbreeding undoubtedly arise during the process of speciation because such blocks have selective value. Behavioral isolation can be the strongest form of reproductive isolation. The clearest examples of the biological species concept in action are provided when two species, which have overlapping geographic ranges, do not interbreed owing to differences in pre-mating behaviors. One species or both species refuse to engage in the mating rituals because their respective stereotyped mating rituals are too different. Species might simultaneously possess both premating and post-mating isolating mechanisms in which case the probability of interbreeding is even more remote. It is these cases that are most interesting from the point of view of behavioral mechanism, as natural selection on behavior may have

played a major role in promoting the process of speciation (Dobzhansky, 1941, Butlin, 1987).

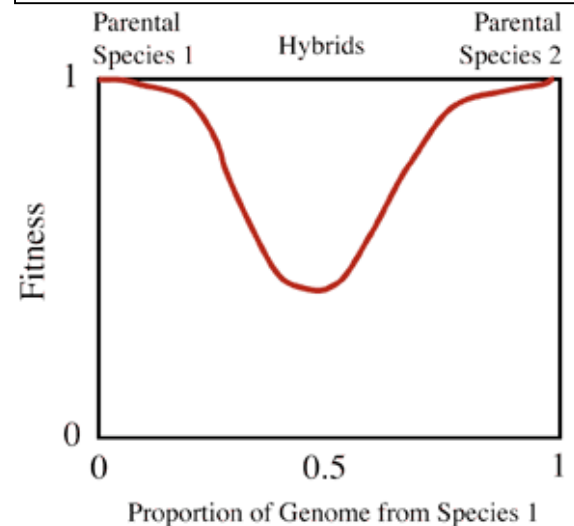
What are the right conditions? This is a difficult question to answer. It requires an understanding of the field of population genetics and the crucial parameter is how much **gene flow** occurs from areas where each semispecies exists in its pure form. A second parameter has to do with the degree of **genetic similarity** between the two semispecies.

Gene flow is the movement of animals between what are referred to as populations of interbreeding animals. Rates of gene flow are usually expressed as the proportion of individuals in a population that move from one population to another generation. If gene flow between the region of contact between the two semispecies and the rest of the semispecies ranges is too high, then the two semispecies will become homogenized into one species even if interbreeding yields relatively low fitness hybrids (Endler, 1977). If gene flow is low enough, then the two pure semispecies will eventually become differentiated into two species owing to the evolution of isolating mechanisms. The evolution of such isolating mechanisms is contingent upon the degree of genetic similarity between the two semispecies.

Large differences in **genetic similarity** between semispecies are more likely to produce unfit hybrids than small differences in similarity. If the two semispecies have many genetic loci that differ in the kind of allele that they possess, then those alleles might not work very well together.

More importantly the way many different genetic loci work together

Figure 5.1. Hypothetical hybrid "unfitness" resulting from an incompatibility between genomes of the two parental species.



may begin to breakdown. The correct operation of one genetic locus (e.g., an enzyme) may require a particular form of the enzyme or protein product that is produced by a different locus (e.g., **epistasis**) (Whitlock et al 1993). These enzymes are most likely to be compatible in their function if they come from the same species. If they are from different species, they may not integrate well with other loci in the organism and a hybrid individual may die. Imagine such genetic interactions occurring at all possible loci (e.g., 10,000 loci in *Drosophila* or 30,000 loci in humans). These multilocus effects can be manifest as **genome-wide gene interactions** that reflect epistasis. The probability of a hybrid being more unfit has to do with how many loci in each semispecies are homozygous for different alleles. **Hybrid breakdown** occurs when many of these loci are fixed for different alleles in the two subspecies and the production of offspring by the hybrids is not possible because an F1 × F1 crosses yields too many incompatible gene-by-gene combinations. N.B. The F1 are heterozygous at all loci for spp. 1 and 2, but the F2 can exhibit any genotype combination between spp. 1 and 2, across all loci in the genome (a phenomenal level of variation).

Genetic similarity drops as we change from the different levels of differentiation, going up the hierarchy of differentiation: populations → races → subspecies → semispecies → sibling species → species. By the time species are compared there is generally little or no gene flow between species. As genetic similarity drops, the differences increase and the possibility of hybrid breakdown increases. A species is thought to have a **coadapted genome** in which many genes are finely tuned by the proper interactions with other genes.

Clearly genetic differentiation is occurring during speciation but it is not enough to define a species. It can vary from organism to organism and among the modes of speciation. In practice, even the criterion of no gene flow between species is not the defining feature of species. As we will see below, the biological species concept begins to break down in areas or at times of speciation or incipient speciation (**semispecies**). In such cases, alleles leak across the hybrid zone from one species into another.

Modes of Speciation Classified by Geography

To understand the origin of species differences it is necessary to consider how genetic differences arise between species. Geographic

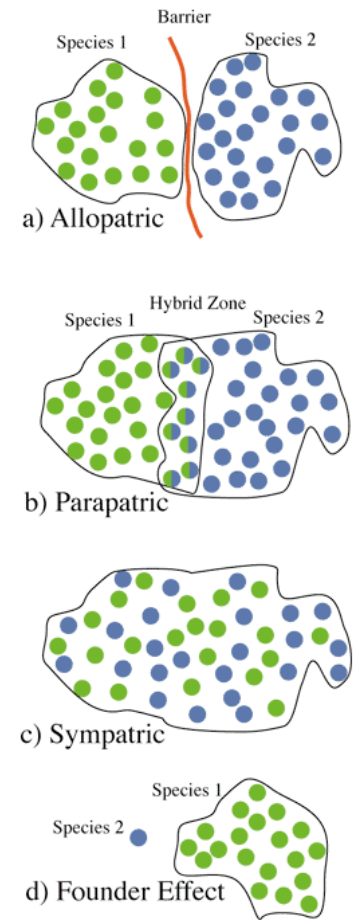
subdivision decreases gene flow. Depending on the population size, genetic drift can play a major role in promoting genetic differentiation.

If population size remains low for a long period of time it is possible for genetic differences to accumulate between geographic areas by the random process of genetic drift. Selection can promote speciation because genetic changes can rapidly accumulate in one area where selection favors certain behaviors and morphology relative to another area where alternative alleles are favored by natural selection. Finally, the amount of gene flow, which is usually described in terms of the movement rate, is a key parameter that influences the rapidity of speciation. By lowering gene flow, subdivision promotes speciation (Endler, 1977). Ernst Mayr (1942) has argued that some form of geographical subdivision is required for speciation.

Most models of speciation relate to the degree of geographical subdivision:

1. **Allopatric Speciation** -- speciation occurs in geographic isolation,
2. **Parapatric Speciation** -- speciation occurs in adjacent populations with gene flow,
3. **Allo-Parapatric Speciation** -- populations are initially divided (allo-) but secondarily come into contact with subsequent parapatric speciation,
4. **Sympatric Speciation** -- speciation in a panmictic population.

Figure 5.2. Models of speciation based on geographical subdivision. Models of speciation relate to the degree of geographical subdivision for Allopatric → Parapatric → Sympatric, which ranges extreme to none. Founder effect speciation is an extreme form of subdivision in species formation takes place in a small isolated population.



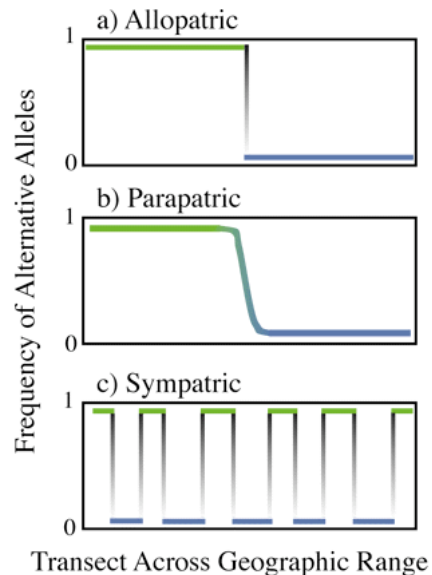
5. **Peripatric Speciation** -- speciation occurs in many populations simultaneously, which have limited gene flow (c.f., Founder).
6. **Founder effect** -- speciation occurs in one event in which a group of founders colonizes a remote location. By virtue of the small size of the founding group the sampling of the gene pool is very limited, leading to genetic drift at the founding event.

Diagnosing Models of Speciation from Clinal Variation

Patterns of variation across the species may provide some basic diagnostic features that distinguish the four modes of speciation. A **cline** is any systematic change in a trait across the range of a species. Clines can be abrupt or gradual. Presence of clines in morphological or behavioral traits is used as evidence for one mode of speciation over another (Fig. 5.3). However, some of the best evidence of **clinal variation** comes from an analysis of gene frequency across the species range. Another parameter that can be used to discriminate the types of

speciation is related to rates of gene flow and how alleles at many genetic loci are distributed across the species range. From such data, we can infer a lot of information about gene flow (Slatkin, 1981). Clines in either genetic, morphological, or behavioral traits are quite useful in diagnosing the probable mode of speciation (Endler, 1977):

Figure 5.3. Hypothetical change in frequency of allele frequency across the range of two species under allopatric, parapatric, and sympatric models of speciation.



1. **Presence** of a cline would suggest a particular mode of speciation has operated in the past. Presence of a geographical barrier at the cline would reinforce the notion that the speciation event was due to allopatric speciation. The change in behavioral

traits, morphological traits, allozymes, or some other form of genetically based variation resembles a step function (see Fig. 3.3). A very abrupt change in traits where the two species come into contact would suggest little or no gene flow in the present day. Lack of gene flow in the present day could be due to the evolution of a reproductive isolating mechanism during an **allopatric speciation** event in the remote past, but by the present day the original barrier has been removed.

2. The **Shape of the Cline** might also be indicative of gene flow. Clines can be gradual across a hybrid zone and span 1000 of km or clines can be very abrupt and occur over a few 100 m (compare Fig. 5.3 A, B). Is the cline maintained by persistent and strong natural selection that favors one form on one side of the cline and another form on the other side of the cline? If yes, then the two species along with their hybrid zone might be a candidate for the **parapatric model of speciation**. In the case of a cline that is being maintained by persistent selection, the cline may have arisen as a primary response to different selection regimes in the species range.
3. **Origin of the cline** or presence of hybrid zone is another attribute that might distinguish modes of speciation. Is the cline due to **secondary contact**? In this case, the two species were in allopatry in the remote past and brought into secondary contact in the present day. Weak gene flow referred to as **introgression** began once the sibling species were rejoined at the hybrid zone. However, complete reproductive isolation did not occur during allopatry, but rather the species differences were **reinforced** once the two species came into secondary contact. The cline is said to have evolved by **allo-parapatric speciation**.
4. **If there is no cline** and the two species can be found co-occurring across a large geographic range, **sympatric** speciation may have produced behavioral isolation. A transect across the range of the two species would show isolated pockets of one pure genotype, or the other pure genotype, with no present day gene flow between the two species (Fig. 3.3C).
5. **A phylogeny** is critical in interpreting the age of the contact and the order of evolutionary events. For these reasons, I introduce key phylogenetic concepts in the examples I discuss below.

Parapatric versus Allo-Parapatric Speciation

Any geographic factor that severely restricts gene flow will allow populations of a species on one side of the barrier to begin to differentiate from species on the other side of the barrier. Given enough time, the genetic differences became large enough to warrant calling the "races" on one side or the other a different species. Allopatric speciation occurs under the following conditions:

1. Isolation of a colony (e.g., island and mainland)
2. Division of species range by extrinsic barrier (mountain range, etc) or extinction of populations in intermediate parts of the range.
3. Isolation by geographical distance.

For a species to arise by **parapatric speciation**, there must be an abrupt discontinuity in the abiotic environment.

Natural selection on one side of the environmental discontinuity favors one set of traits compared to the traits favored on the other side. If selection is strong enough it leads to disruptive selection in any populations that are located at the abrupt transition where parapatric speciation may be occurring. The fitness of an individual is determined by the fraction of genes from each parental type. Individuals that are pure for each parental type will tend to have higher fitness than individuals with a genome that is formed from an admixture of the two parental combinations. These hybrids come from crosses between the two parental forms. However, animals that interbreed (e.g., mate with a different type) at such **hybrid zones** tend to show underdominance in fitness, that is their progeny the heterozygotes (hybrids) have lower fitness. Another way to think of the pattern of parapatric speciation is to view it as disruptive selection against individuals that are heterozygous for a large number of loci. The genes in the heterozygous condition have lower fitness than homozygous configurations because of the strong environmental differences on either side of the contact zone.

If there is any viability of the hybrids, alleles may leak across and introgress across the zone. Leakage of alleles across the hybrid zone is referred to as **Introgressive hybridization**. Some alleles may spread quite far across the zone whereas others will be quite abrupt and coincide with the hybrid zone.

Can we distinguish between primary and secondary contact? In principle, we cannot distinguish between primary and secondary contact, which is why the models of speciation are so fiercely debated. However, a phylogeny helps in dating contact zones.

The process of speciation is by its very nature a historical process (Endler, 1977). The origin of the cline is a key component of all models of speciation, and this event can only be inferred from the patterns of clinal variation that we see across the present-day species ranges. We see many fully formed species. While we also see incipient species in the process of formation the actual cases are difficult to resolve as allopatric versus parapatric speciation, because events have occurred in the remote past. Nevertheless, studies of the geographic pattern of races in a species and their likelihood of interbreeding have provided us with many splendid examples of apparent allopatric speciation. Nothing is more challenging to the imagination than visualizing a special process of allopatric speciation that produces a **ring species**.

Ring Species in Gulls?

Ring Species arise from a peculiar form of allopatric speciation that takes place when the center of a species range is unoccupied because the habitat is unsuitable. Adjacent races all around the ring will interbreed, however the races at the termini are so divergent, that there is no interbreeding, and they can exist in sympatry without interbreeding.

Species of sea gulls around the Arctic Ocean have been posited as a classic example of a ring species. The polar ice cap limits the species range of Sea Gulls to a circumpolar ring (Geyr von Schweppenburg 1938, Mayr 1942). According to Mayr's (1942) hypothesis, there are a number of races of sea gulls as one circumnavigates arctic circle. Races from America interbreed with races from Europe. Races from Siberia interbreed with races from America. Races from Siberia freely interbreed with races from the Caucasus. However Central European races do not interbreed with those from Western Europe. All along the ring there is supposedly gene flow, but where the two ends of the ring meet in Europe there is no gene flow. The ring species of gulls seems to form a cline in that there are small changes in coloration and behavior of the gulls along the length of the ring. However, not enough mate discrimination occurs between adjacent populations until the ends of the ring meet in Europe.

However detailed molecular analysis of the phylogenetic relationships among sea gull clades reveals two refugia, one in the Atlantic and one in the Caucasus (Figure 5.4). These reflect an very ancient split as revealed by reconstruction of the phylogeny, thereby invalidating Mayr's classic hypothesis. Any inference of speciation requires detailed analysis of evolutionary ancestry. I defer a formal discussion of these methods until Chapter 18. These gene trees are conceptually similar to genealogical relationships for members of a family. The gene tree for gulls is simply too deep to support Mayr's (1942) elegant hypothesis of a ring species. Rather the tree indicates that isolation and the allopatric mode of speciation led to the differentiation, after which movement from the refugia promoted secondary contact (Fig. 5.4). The survey that invalidated the gull ring species is solid. However, the geographic survey also suggests that the ring might be closing in North America, as lesser black gulls spread east from Greenland. There appears to be a new research opportunity on speciation unfolding before our very eyes.

A Paint Experiment in Gull Species Recognition

Work by Smith on a different species of sea gull suggests that the behavioral isolating mechanism, which operates in gull species recognition, may be quite simple. Smith investigated the signal that is used to isolate the two species into *Larus hyperboreus*, the glaucous gull and *Larus thayeri*, Thayer's Gull. The glaucous gull has a yellowish eye ring, and Thayer's Gull has a purple eye ring. Smith painted the eye rings of Thayer's males yellow, and this allowed Thayer males to successfully pair with glaucous females. However, males would not mate with glaucous females until Smith painted the glaucous females purple. Such paint experiments are a powerful means of demonstrating the role of signals in species recognition. Unfortunately, recent studies seem to have invalidated Smith's earlier work and a careful auditing of Smith's research notes, sadly reveals a case of scientific fraud!

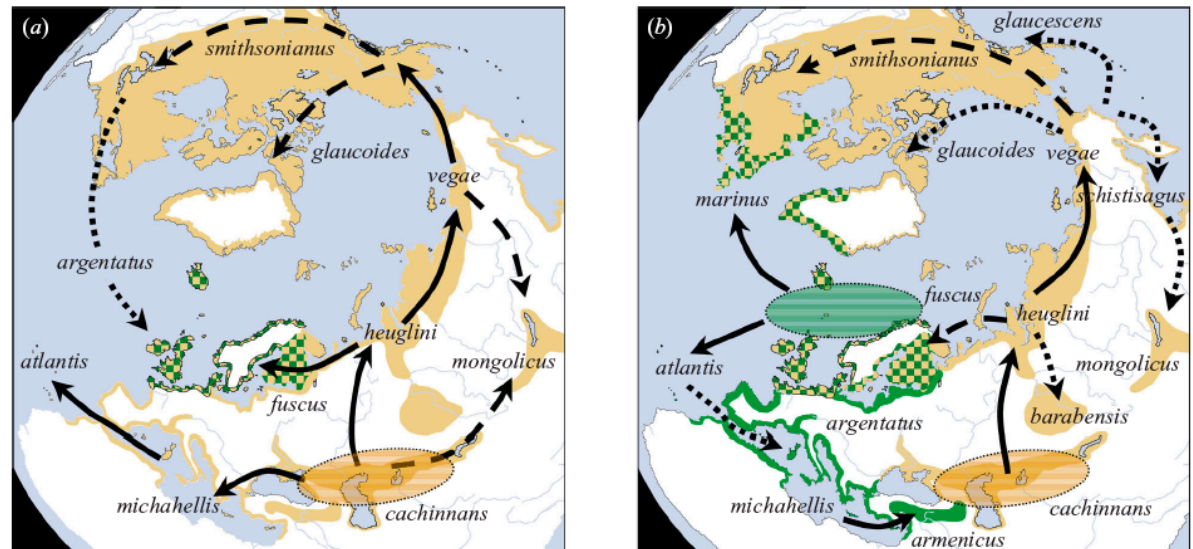
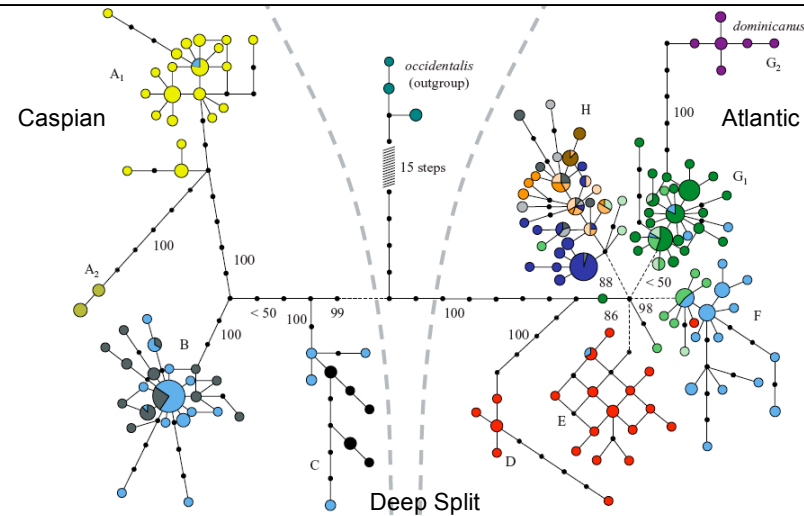


Figure 5.4. Top panel: Two hypotheses about the history of the herring gull complex. Large ovals show hypothetical refugia. Arrows indicate inferred colonization routes, with temporal progression from ancient to most recent events indicated by solid, broken and stippled arrows. (a) The ring-species model of Mayr assumes a single Aralo-Caspian refugium and a recent invasion of gulls from North America to Europe, where they now overlap with lesser black-backed gulls (checkerboard pattern). (b) Alternative model based on a *phylogeny* for gulls (**Bottom Panel**) indicates 2 ancient refugia. Current ranges of taxa derived from the Atlantic refugium are shown in green; those derived from the Aralo-Caspian refugium are shown in pale ochre; checkerboard pattern shows areas of overlap. *Larus marinus* developed reproductive isolation in allopatry (probably in northeastern North America) before making secondary contact with North American *smithsonianus* and Eurasian *argantatus* and *fuscus*. Two separate colonization events from the Atlantic into the Mediterranean led to differentiation of *armenicus* and, much later, *michahellis*. (Liebers et al. 2004).



Speciation in a ring based on isolation by distance

Despite rejection of the ring species hypothesis for gulls, are there other examples of ring species? The two critical criteria for a ring species are:

- 1) Isolation by distance around the ring (as the species spread from some refugia,
- 2) After this range expansion, when the termini of the ring meet species do not interbreed by some isolation mechanism, which in the case of behavioral trait, involves a recognition signal.

Irwin et al (2001, 2005) have recently elucidated a new ring species, which satisfies these two critical criteria. Two species of Phylloscopine warbler, *Phylloscopus trochiloides viridanus* of western Siberian and *P. t. plumbeitarsus* of eastern Siberian, contact north of the Himalayas. Where the two subspecies meet there is an abrupt discontinuity in genetic variation (Fig. 5.5), but along the length of the ring there is a clear pattern of isolation by distance (Fig. 5.5).

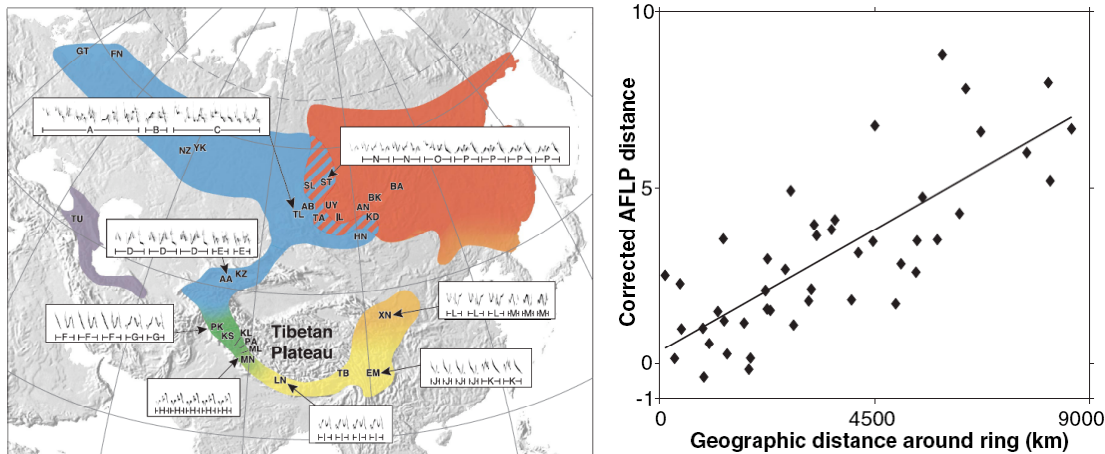
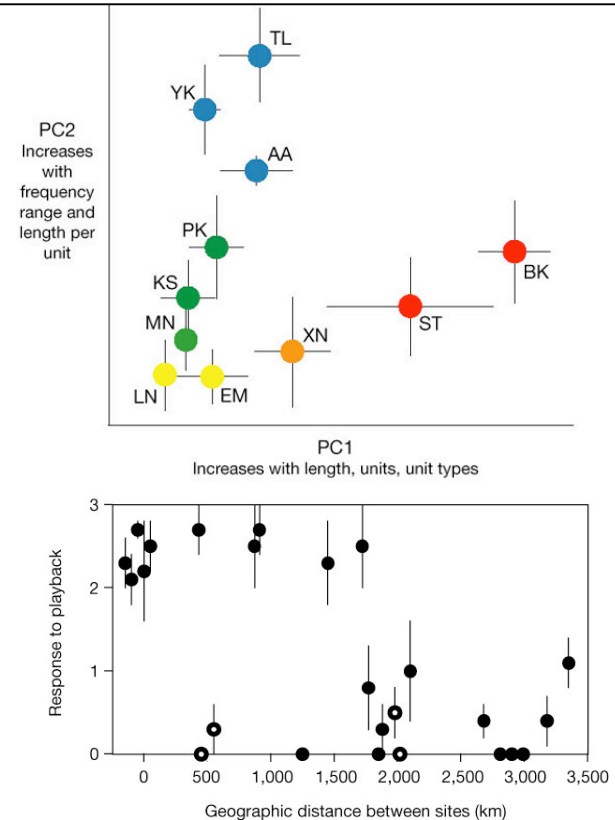


Figure 5.5. Left panel: Map of Asia showing the range of greenish warblers in the breeding season along with sonograms of male songs along the ring. Different colors represent different subspecies (*P. t. viridanus*, blue; *ludlowi*, green; *trochiloides*, yellow; *obscuratus*, orange; *plumbeitarsus*, red; and *nitidus*, violet). Colors grade together in areas of gradual morphological change. The hatched area in central Siberia indicates the overlap zone between *viridanus* and *plumbeitarsus*. The gap in the ring in northern China is likely due to recent habitat destruction. **Right panel:** An analysis of the pairwise genetic distance among all of the populations along the ring indicates that genes grade smoothly along southern portions of the ring in each arm of the distribution with an abrupt change from 0 to 9000 (ring terminus) (From Irwin et al. 2001, 2005).

The other critical piece of evidence is the male signal, which is song, and the female response to divergent male songs that have evolved along each arm of the northern ranges of *P. trochiloides* (Figure 5.6).

Figure 5.6. Upper panel -- Geographic variation in the song of the greenish warbler as quantified by principal components analysis. Shown are population means and standard deviations. Songs distinctly differ between *viridanus* (blue) and *plumbeitarsus* (red), but change gradually through populations to the south. Both PC1 and PC2 are axes of complexity, and Himalayan populations (yellow and green) have the simplest songs (low PC1 and PC2). **Lower panel --** Relationship between geographic distance and song recognition. The vertical axis shows the response to recordings. Open circles are playbacks of *viridanus* recordings to *plumbeitarsus* populations or vice versa, and filled circles are all other experiments. The cluster of points at a geographic distance of zero represent trials in which songs recorded in a population were played to birds in that population. Generally, birds respond strongly to recordings from populations up to 1,500 km away, but *viridanus* and *plumbeitarsus* across Siberia do not respond to each other even when only 500 km apart. (Irwin et al. 2001).



Ring Species in Salamanders and the Evolution of Mimicry

A ring species of salamander, *Ensatina eschscholtzii*, is found in higher altitude regions of California such as the Sierran and Coast Ranges, but is conspicuously absent from the great Central Valley of California. The donut of the ring is the arid central valley. Abundant evidence of gene flow along the circular cline is provided by comparing the frequency of electrophoretic alleles in adjacent populations of the salamander. Gene flow is present throughout the coast range from south to north, from the coast range to the range that crosses northern California to meet with the Sierras, all down the length of the Sierras, and from the Sierras to the transverse ranges of southern California. Gene flow abruptly ends in a sharp dividing line located in southern California. The ring of gene flow that traverses the two thousand-mile perimeter of California finally expires and speciation appears to have run its course (Wake, 1997).

The ring species *Ensatina* is noteworthy in terms of its morphology and behavior. Where *Ensatina* co-occurs with the California Newt in the coast range, *Ensatina* has evolved a color pattern that resembles the toxic Newt in several traits. It has a reddish cast to its belly and limbs, and its eye is colored a brilliant yellow, both are thought to be features important for warning coloration in the newt, which this form of *Ensatina* mimics. We will take up the causes of **warning** or **aposematic coloration** in chapter 14, which treats predator and prey interactions, but we briefly introduce basic concepts with the *Ensatina* ring species. In other areas *Ensatina* is cryptic. Because the forms are so different, I will briefly describe each morphospecies, their distribution, and whether they are cryptic or mimetic species. In addition, two different forms of crypsis are exhibited by *Ensatina* (Ruxton et al. 2005, Stebbins 1985):

- 1) **Disruptive coloration** which breaks up the body outline
- 2) **Background matching** where color and/or pattern match substrate.

While the ring species idea still has merit, David Wake (1998) has described a number of interesting episodes of allopatry and secondary contact. Nevertheless, behaviors limit gene flow at contacts. Around the ring, different forms come into contact (mimetic, disruptive coloration, background matching), and at the contact zones between types, gene flow is likely to be restricted because of ongoing selection against hybrids produced from crosses between any given anti-predator type.

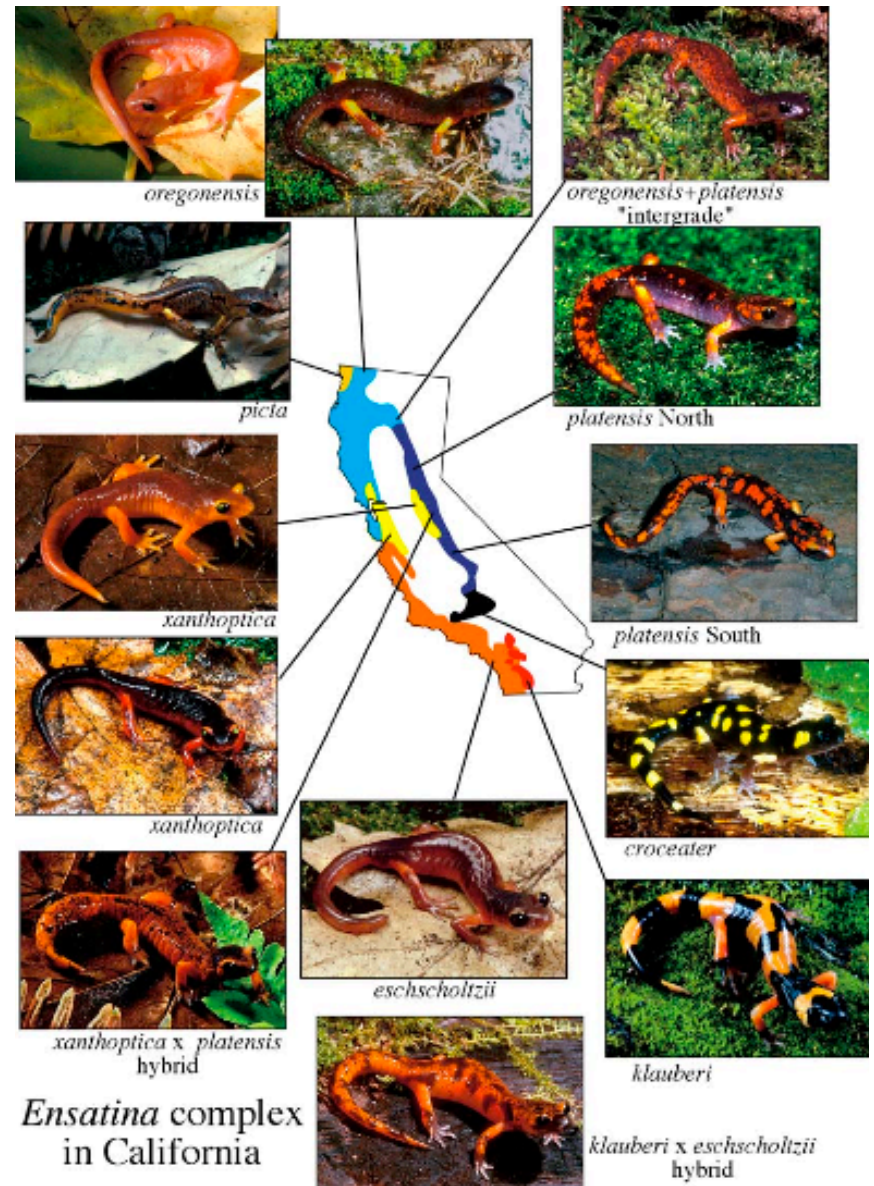


Figure 5.7. The *Ensatina* ring species complex in California along with hybrid forms. Blotched subspecies are distributed along the inland axis, and unblotched subspecies are distributed along the coastal axis. One of the forms, *Ensatina e. xanthoptica* is a Batesian mimic of newts (*Taricha*) (Wake 2006).

Blotched *Ensatina* may disrupt the outline of the body

Blotching in animals is thought to function in crypsis on dark substrates

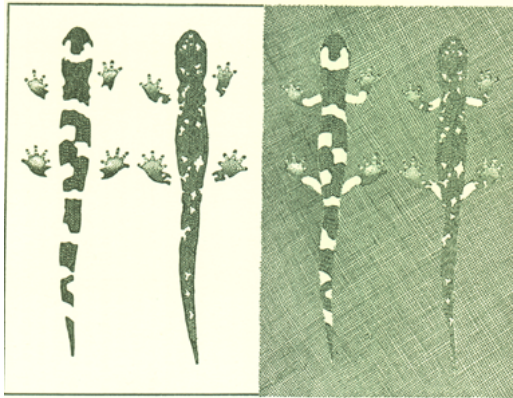


Figure 5.8: From Stebbins (1949, p 494). Large blotches (*klauberi*) break up the body outline on dark or light backgrounds, but small blotches (*platensis*) only break up the body outline on dark backgrounds. These reflect two fundamental strategies of crypsis in animals (Ruxton et al. 2005):

- 1) Break-up the outline
- 2) Background matching.

by breaking up the body outline

(**disruptive**

coloration). *Ensatina*

eschscholtzii *platensis*,

which is distributed in the

Sierra Nevada Mountains,

exhibits dark brown dorsal

coloration with irregular

orange blotches. There is a

hybrid zone with *E. e.*

xanthoptica populations,

which have colonized the

Sierra Nevada foothills

from the San Francisco Bay

area (Wake et al. 1989).

This colonization occurred

during the glacial periods

of the Pleistocene when the

valley was cool and wet.

Ensatina e. croceator (E),

which is distributed in the

northern Transverse ranges of southern California, exhibits black dorsal coloration with yellow blotches. Blotches are larger relative to *platensis*.

Ensatina e. klauberi, which is distributed from the Transverse Ranges of southern CA to the peninsular ranges of northern Baja California,

Mexico, exhibits a black background with yellow or cream blotches. Of the blotched subspecies, blotches are largest in *E. e. klauberi*. Blotches

are thought to be cryptic via disruptive coloration on high-contrast backgrounds, and functional on light or dark substrates. *Ensatina e.*

klauberi is sympatric with the unblotched subspecies *E. e. eschscholtzii*,

with little or no interbreeding (Wake et al, 1986) (Figure 5.7).

Unblotched *Ensatina* may match the background

Ensatina e. picta, which is restricted to northern California and southwestern Oregon, exhibits small dorsal patches of black melanophores and yellow lipophores. The color pattern is considered

cryptic by background matching in the dark Redwood forests it inhabits.

Ensatina e. oregonensis, which is distributed from central British Columbia, Canada south to northern California, exhibits a dark dorsum,

flesh colored ventrum, light yellow proximal limbs, and yellow speckles in the eye. This color pattern is also thought to be cryptic (Stebbins,

1949). In California, it meets *E. e. platensis* in the northern Sierra Nevada, and *E. e. xanthoptica* in the San Francisco Bay Area. *Ensatina*

e. eschscholtzii, which is distributed from Monterey Bay to San Diego, exhibits a reddish-brown dorsum, a black eye, and a fleshy-white ventral

region. The eye is solid black, unlike *E. e. xanthoptica* and *E. e.*

oregonensis. This color pattern is considered cryptic (Stebbins, 1949).

An *Ensatina* that mimics the toxic Newts

Ensatina eschscholtzii xanthoptica, which is distributed north, south and east of San Francisco Bay, has a brown dorsum, a striking orange

ventrum, orange eyelids, orange proximal limb segments, and bright-

yellow iris patch. The pattern exhibited by *E. e. xanthoptica* is

hypothesized to be mimetic of newts (family salamandridae) (Stebbins,

1949; Brown, 1974, Wake et al, 1986). Two newt species, *Taricha*

granulosa and *T. torosa*, co-occur with *E. e. xanthoptica*, have a very

similar color pattern (brown dorsum, orange belly, and yellow eye

patch), and are among the most poisonous animals in the world because

they possess large amounts of the neurotoxin tetrodotoxin (Brodie et al.

1974, see Chapter 3 for a discussion of the molecular biology of the

arms race). There is sufficient tetrodotoxin in some populations of *T.*

torosa near San Francisco bay to kill 1,200-2,500 20 g. mice (Brodie et

al. 1974). Orange and yellow function as **warning or aposematic**

coloration in newts (Johnson & Brodie 1972; Johnson & Brodie 1975;

Brodie 1977). These colors also function in mimicry in *E. e. xanthoptica*

(Stebbins, 1949; Wake et al, 1989), which Kuchta (2005) proved with

clay model experiments (discussed in Chapter 14 on anti-predatory

strategies). In feeding trials, *E. e. xanthoptica* is edible to scrub jays

(Kuchta and Sinervo unpub. data), but the color does cause scrub jays to

pause before feeding, relative to cryptic prey like *E. e. oregonensis*.

Co-evolutionary interactions between toxic Newts and Aposematic

mimics are a common form of evolutionary cheater. The red-spotted

newt, *Notophthalmus viridescens*, in the eastern USA, is as toxic as *T.*

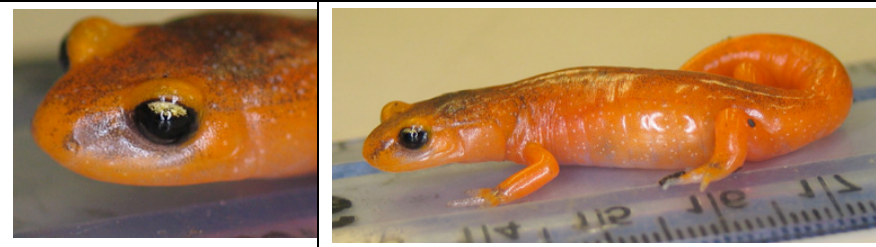
granulosa, and is mimicked by the red salamander, *Pseudotriton ruber*

(Howard & Brodie 1971; Pough 1974; Brandon & Huheey 1975).

Figure 5.9. *Ensatina eschscholtzii xanthoptica* at a Sierran hybrid zone.



F1 hybrid collected from the hybrid zone



Ensatina eschscholtzii platensis at a Sierran hybrid zone.

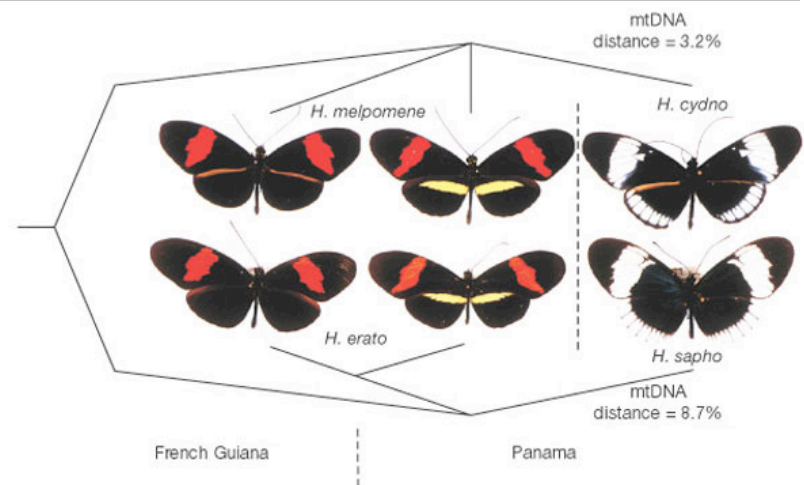


When the Aposematic mimic, *E. e. xanthoptica*, hybridizes with a cryptic form like *E. e. platensis*, spectacular fluorescent orange hybrids are created. Do predators find these hybrids as conspicuous, and eat them at a higher rate than the pure types, thereby generating a key force required for speciation – hybrid unfitness? We tested this hypothesis with clay model experiments in which we (Kuchta, Sinervo and the Behavioral Ecology class of 2005 and 2007) made 100's of clay models and painted them the color of the cryptic type, aposematic type and a hybrid type with an orange dorsal stripe (c.f. orange on dorsum of hybrid, Fig. 5.9). We found that the pure types of models had higher survival than a hybrid type in the contact zone, confirming our hypothesis that hybrid forms are unfit in terms of anti-predatory signals.

Speciation in Müllerian mimics

The previous example of an **aposematic mimic** is also referred to as a **Batesian mimic** because of the pioneering work of Bates, a 19th century naturalist who while working in Central America, discovered a mimicry complex involving a species, which is toxic or unpalatable, being mimicked by a non-toxic form. I prefer the term **aposematic mimic** because, being forgetful, I can never keep the two terms used to describe mimics straight. The other kind of mimic is referred to as an **Müllerian mimic** in the case of a group of species that resembles one another, but all are unpalatable or toxic. This term is named in honor of Müller, who working in South America, discovered a complex of unpalatable or toxic mimics, which had evolved in parallel to self-resemble each other. My preference is to refer to these types as **self-reinforcing mimics**, because the term refers to the process of selection that gives rise to the types. We will encounter more rationales for adopting the terms I describe in Chapter 14 on anti-predator strategies. The phylogeny for Müllerian species of *Heliconius* butterfly from Central America, presents a clear example of parallel speciation in which new aposematic forms have evolved. Each of the 3 signal types has been independently derived in a “twin species” that helps to self-reinforce its distinctive signal (white bars – the ancestral-most condition, red bars, and red bars with yellow).

Figure 5.10. The sister species *H. melpomene* and *H. cydno* are sympatric in Central America and the Andean foothills, where they differ in mimicry. They occasionally hybridize and backcross in nature but hybrid females are sterile.



I wanted to include an example of speciation in which the mimicry complex is due to the process of Müllerian or self-reinforcing mimicry. This example along with the example of aposematic mimicry and speciation are clear examples of socially mediated speciation (broadly defined to include the species level of selection). I discuss the subject of socially mediated speciation in Chapter 19 on social evolution. Suffice it to say that speciation by this process occurs in the domain of **Hamiltonian social interactions** (outlined in Chapter 4) of **altruism, mutualism, competition** or **spite**. In brief, Müllerian mimics can be viewed as greenbeards that act between species. Müllerian mimics share altruistically the costs of evolving anti-predators strategies. Predators make mistakes and kill unpalatable forms, but evolve over time to avoid Müllerian mimics. The mimics exhibit a form of co-evolutionary cooperation. The signal is also self-reinforcing within a Müllerian type.

Throughout most of its range, *H. melpomene* mimics the black, red and yellow pattern of *H. erato*, whilst *H. cydno* mimics the black and white pattern of *H. sapho*. *Heliconius cydno* and *H. melpomene* last shared a common ancestor about one million years ago (Fig. 5.10). In contrast, *H. sapho* and *H. erato* evolved more recently. This phylogenetic evidence implies that *H. cydno* and *H. melpomene* have diverged to mimic *H. sapho* and *H. erato*, rather than vice versa. The relevant comparisons for speciation are to be made among types that are sympatric in Panama (right side of phylogeny, Figure 5.10), not allopatric forms in Guiana.

In the first series of test, Jiggins et al. (2000) carried out simple mating trials among males of the two different forms of *H. melpomene* (i.e., simple red, red+yellow) and *H. cydno*. *Heliconius* females mate soon after eclosion, when they are unable to reject males (McMillan et al. 1997), so that courtship and assortative mating is largely due to male choice, which is counter to the typical pattern of female choice (Chapter 3). They found that each color type from the critical area of sympatry in Panama tends to strongly court females of the correct type (Fig. 5.11). The exception in Fig. 5.11 is *H. melpomene* from Guiana, which cannot discriminate very well between the red form and the red+yellow form, but it actually has no “evolutionary experience” with the red+yellow form, thus speciation processes have not had opportunity to evolve pre-mating isolation in males. This comparison of mate preference from an area of allopatry in Guiana serves as a very useful control for the preference trials among types from the Panamanian area of sympatry.

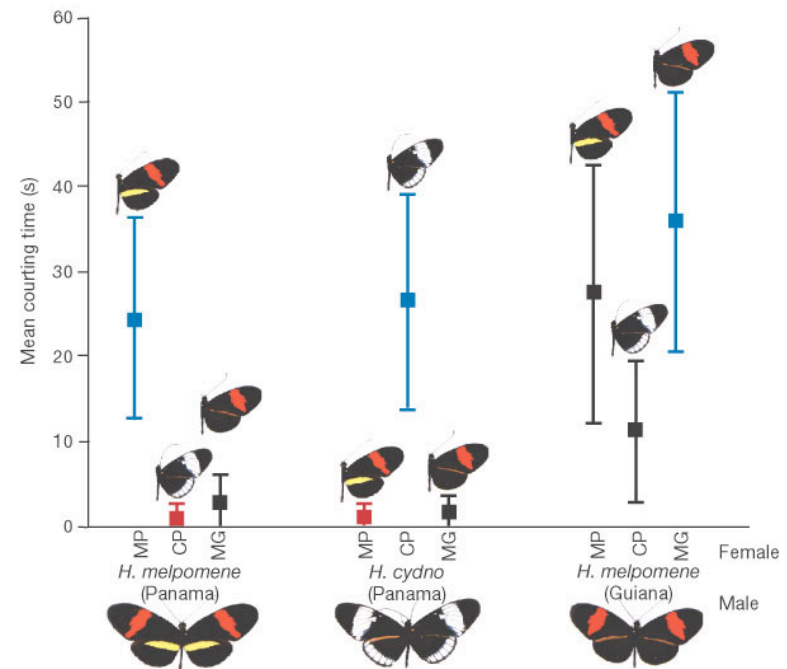
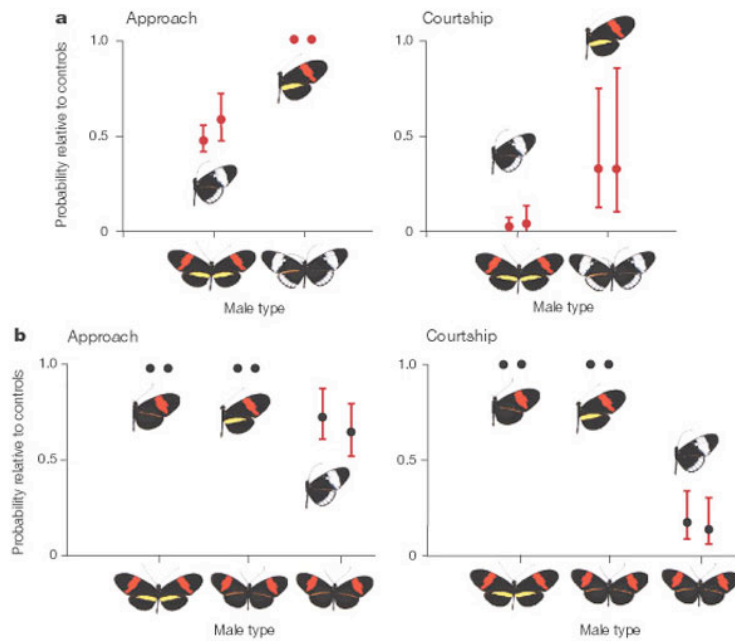


Figure 5.11. Mate preference trials among color types of sympatric *Heliconius* butterflies. Notice that excellent mate discrimination of males is present in the two sympatric species from Panama (*H. melpomene* and *H. cydno*), but the *H. melpomene* from Guiana, with no prior evolutionary experience at discrimination, is confused and attempts to court the wrong species (Jiggins et al. 2000).

Rather than use live females in courtship tests, Jiggins et al. (2000) ingeniously used paper models (and real wings) that were made to move. They tested males from each of the color types to determine what components of species recognition were due to color *per se*, compared to other potential factors in mate choice like pheromones.

Heliconius melpomene males from Panama approached *H. cydno* color patterns about half as frequently as those of their own type, and were much less likely to court them (Fig. 5.12). Similarly, *H. cydno* males were a third as likely to court a *H. melpomene* pattern that is colored red as their own type that is colored white. However, the probability of initial approach by *H. cydno* to its own type did not differ from that towards other species (Fig. 5.12). Jiggins et al. (2000) suggest that this initial attraction of male *H. cydno* to the red may be due to a generalized attraction to red flowers of the plant *Heliconius*, which the butterflies use as a food source (they are in fact named for this genus of passion

Figure 5.12. Comparisons between Panama populations (sympatry) (a) and the Guiana population (allopatry) (b). Values are estimated relative to within-race controls (equal to 1 in each case). Paired data points for experiments using real wings (left) and colored paper models (right) are shown for each comparison.



flowers). Butterflies clearly respond to visual cues. Once again, *H. melpomene* males from Guiana, were confused when presented with models with which they had no co-evolutionary experience (Fig. 5.12).

Males from all color types of *H. melpomene* showed greater discrimination between live females (Fig. 5.11) than between models (Fig. 5.12), indicating that cues other than color, such as pheromones, may be involved. In summary, *H. melpomene* males sympatric with *H. cydno* discriminated more strongly than *H. melpomene* allopatric to *H. cydno*. This pattern is expected if mate preference has been 'reinforced' to prevent the production of unfit hybrid offspring in sympatry. As in the case of hybridization of different salamanders, the hybridization of different color types in a Müllerian mimic are very strongly selected against because the hybrids are outside of the "search domain" that is self-reinforced by Müllerian mimicry. Birds mistakenly kill such hybrids quite efficiently, even if they are distasteful or toxic (Chapter 14). The concept of **reinforcement** in speciation is discussed next, since it is the process by which behavior evolves to refine pre-mating isolation.

Sympatric speciation

Sympatric speciation is the origin of two new species with the two forms in the same place. Conditions for speciation by sympatric speciation are very stringent. Gene flow between incipient species must be eliminated, or curtailed, otherwise reproductive isolation is not possible. Isolation by geographic distance as is seen in allopatric and parapatric speciation can limit gene flow and lead to the process of speciation. However, any behavioral changes that have occurred in isolation are really a secondary consequence of the geographical subdivision. When secondary contact is achieved, behavior only serves to reinforce the initial reproductive isolation achieved by distance, genetic drift, or natural selection. Achieving sympatric speciation in which individuals are found in the same geographic locale is a challenge. Behavioral processes must play a direct role in such forms of speciation. However, controversy surrounds all theories of sympatric speciation that involve a gradual speciation event because sibling species, which are panmictic, or which readily interbreed across the range, will not speciate.

A key aspect of the theory of sympatric speciation involves the process of natural selection that favors the evolution of female discrimination and divergence of male traits that females use for mate discrimination. Dobzhansky (1941) suggested that natural selection should favor the more finely tuned discrimination mechanisms of the female, in response to the reduced viability that results from hybridization (see Fig. 5.1). Thus, a key component of this theory is some form of disruptive selection that selects against hybrid phenotypes, which then favors the evolution of mate discrimination mechanisms. A process referred to as **reproductive character displacement** should accentuate differences in courtship behavior or mate preference in sympatric populations of the two incipient species compared to the differences between allopatric populations of the two incipient species. Butlin (1987) suggested that the term **reinforcement** be reserved to describe evolutionary interactions between incipient species, in which hybrid unfitness promotes divergence in courtship behavior or mate preference in sympatric compared to allopatric populations.

The evolution of such divergence, in the face of gene flow is a tremendous challenge for the evolution of species by the process of sympatric speciation. In a classic population genetic analysis,

Felsenstein (1981) showed that any process of sympatric speciation is unlikely. He modeled this process in terms of 1) the phenotypic attributes that give rise to hybrid unfitness, and 2) the genes that give rise to mate discrimination. Generally, the genes for fitness, are not expected to be linked (either by pleiotropy or by physical linkage) to the genes that give rise to mate discrimination. This key fact places a tremendous genetic constraint on speciation by reinforcement. Every generation segregation of chromosomes will lead to parental species that lack the correct discrimination alleles. Even if the genes for fitness and mate discrimination are linked on the same chromosome, recombination during meiosis is a powerful enough force to dissolve away such associations and eliminate the possibility of sympatric speciation.

Polyploidy

Despite the apparent difficulties of many theories of sympatric speciation, one form of sympatric speciation is universally accepted -- the **instantaneous** mode in which changes in ploidy lead to instantaneous reproductive isolation. While extremely common in plants, it is less common in animals. However, well-documented cases are found in the lower vertebrate classes: fish, amphibians, and reptiles.

For example, Gerhardt (1982, 1994ab) has identified character displacement in male calls where species of *Hyla* tree frogs have come into contact. In this case, the formation of tetraploid (4N) species, *Hyla versicolor*, from the fusion of two diploid parents of *Hyla chrysoscelis* (2N) has led to a new tree frog species. Individuals can only successfully breed with another individual that has similar ploidy (Gerhardt 1982). The fusion of gametes from a diploid parent and tetraploid parent produces triploid offspring, which die during larval stages, and the few hybrids that reach adult size are sterile (Johnson, 1963). While production of triploids can be produced in the laboratory, triploids are rarely found in natural populations of *Hyla* tree frogs (Gerhardt 1982).

Because diploid parental species instantaneously achieve reproductive isolation from the tetraploid daughter species, sympatric speciation is easy to achieve. Indeed Gerhardt and his colleagues have found that the production of new tetraploid species has occurred several times in this species complex. Diploid populations of *Hyla chrysoscelis* probably gave rise to tetraploid populations of *H. versicolor* (Tymowska 1991), though direct evidence is lacking.

Character Displacement in Mate Calling and Female Preference

Gerhardt (1994, 2005) has tested female preferences for male call frequencies of the two species of *Hyla chrysoscelis* frogs in areas of sympatry and allopatry. Males of each species from areas of sympatry are more divergent in key aspects of their calls compared to males from areas of allopatry. In areas of sympatry *Hyla chrysoscelis* males produce a longer call than co-occurring *H. versicolor*.

The key test of character displacement via processes of reinforcement involves assessing whether females in the areas of sympatry have evolved more fine scale preferences than females in areas of remote allopatry. The hypothesis of character displacement would suggest that females from sympatric populations show stronger preferences for acoustic stimuli of sympatric male calls than females from more remote populations. Gerhardt has carried out several phonotaxis experiments to test whether females in allopatry have evolved more fine scale discrimination of the species differences in mate call. He created synthetic calls, which allowed him to vary specific attributes such as call length rather than the fine-scale aspects of the song.

Females do not discriminate against synthetic calls (Figure 5.13), validating the synthetic

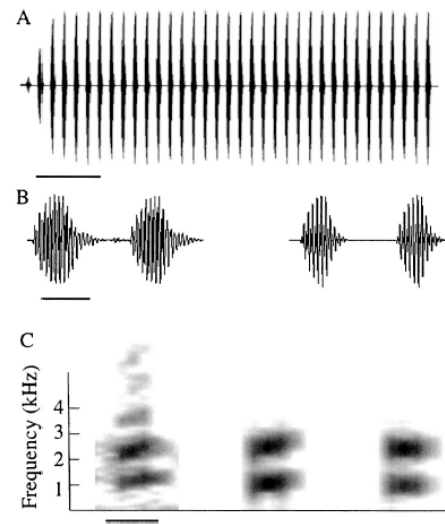


Figure 5.13. Oscillogram of a call by *Hyla chrysoscelis*; time bar indicates 50 msec. (B) Oscillograms of a representative call by *H. chrysoscelis* (left) and a synthetic call of *H. chrysoscelis* (right); time bar indicates 10 msec. Notice that the pulse duration and interpulse silent intervals were about the same. Natural and synthetic signals were equally attractive to *H. chrysoscelis* females in two-speaker tests. (C) Sonograms showing frequency-time profiles of a pulse from natural call of *H. chrysoscelis* (left), a synthetic call that was tested against the natural call (middle), and a synthetic call in which there was no frequency modulation (right); the time bar indicates 10 msec. Females did not show a preference for the synthetic call with frequency modulated pulses to the synthetic call with pulses of constant frequency (From Gerhardt 2005).

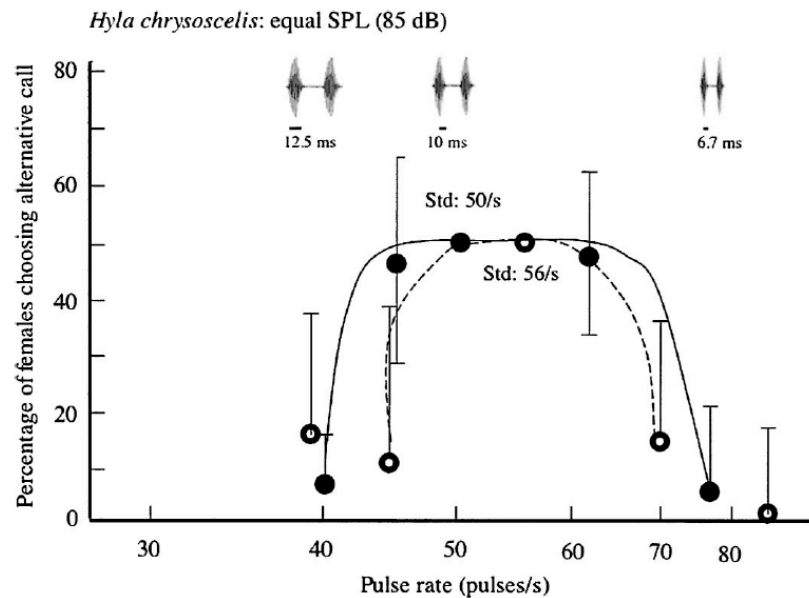


Figure 5.14. Results of two-speaker playbacks to females of *Hyla chrysoscelis* in which different standard calls (solid circles, 50 pulses/ sec; open circles, 56 pulse/sec) were tested against alternatives (same symbols as standard calls) with a different pulse rate. The sound pressure level (SPL) of alternatives was equalized at 85 dB at the release point. The error bars are 95% credible intervals for the proportion of females choosing the alternative stimulus (see the text). The oscillograms at the top show two successive pulses from synthetic calls with different pulse rates, indicating the concomitant differences in pulse duration and interpulse silent intervals. s, second; ms, millisecond (Gerhardt 2005).

call methodology. He then played songs from two speakers. One speaker broadcast a short synthetic call typical of the males of the female's area, the other speaker played a male with longer synthetic calls. Females from areas of sympatry were much more likely to discriminate the correct conspecific short song than females from more remote areas of allopatry. In some cases the discrimination ability was 300% higher than females in remote allopatry.

More recent studies by Gerhardt (2005) have mapped each species acoustic preference function in the area of sympatry (Figure 5.14, 5.15). The two preference functions are centered on dramatically different pulse rates of the male's song, which do not overlap (40-80 pulses/s for *Hyla chrysoscelis* and 15-30 for *H. versicolor*). The **receiver preference function** of each species naturally corresponds to frequencies that males

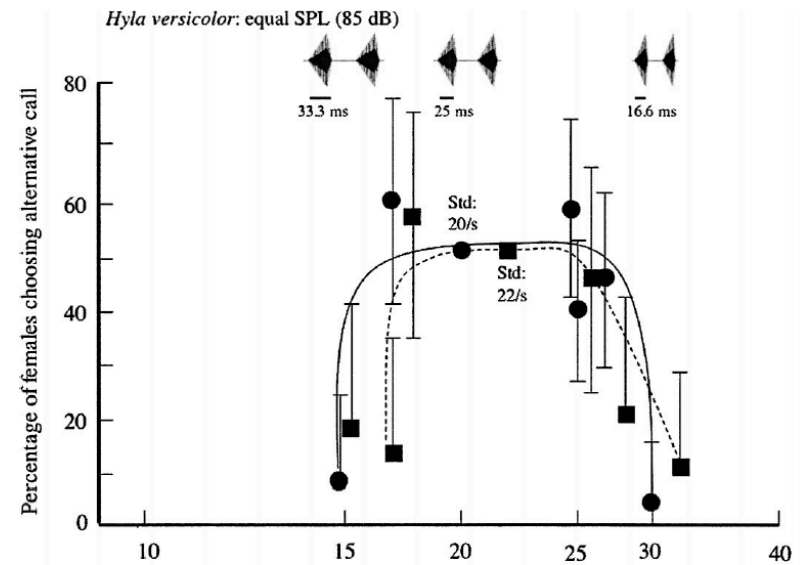


Figure 5.15. Results of two-speaker playbacks to females of *Hyla versicolor* in which a different standard calls (solid circles, 20 pulses/ sec; solid squares, 22 pulses/sec) were tested against alternatives (same symbols as standard calls) with different pulse rates. The sound pressure level (SPL) was equalized at 85 dB at the release point. See the legend of Figure. 4 for explanations for error bars, lines indicating the shapes of the functions, and sample oscillograms. s, second; ms, millisecond (Gerhardt 2005).

of each species emit during chorusing. At the same temperature, males that produce calls with high pulse-repetition rates (i.e., pulse rates) are the diploid species ($2N = 24$; *H. chrysoscelis*); males that produce calls with low pulse rates are the tetraploid species ($2N = 48$; *H. versicolor*; Wasserman 1970). Preference functions elucidated by Gerhardt (2005) reflect the most comprehensive preference functions for any species that communicates by sound (Wagner 1998; Gerhardt and Huber 2002).

Gerhardt's studies are also exemplary for illustrating the role of ploidy in generating hybrid unfitness, which appears to be more common in amphibians than other vertebrates. Other instantaneous modes of speciation are common in other lower vertebrates like reptiles and fish. In the next example, it is clear that parthenogenesis also generates an instantaneous block to speciation.

Production of Asexual Parthenogenetic Species

The production of parthenogenetic species in lizards is quite common. For example, species of *Cnemidophorus* or whiptail lizards from the southwestern deserts have repeatedly produced daughter species by polyploidy. Not only have sibling polyploid species been produced, but reproductive isolation is achieved by another interesting evolutionary event -- many of the daughter species that are produced are asexuals which means they are all female and do not require males to produce offspring. The evolution of an asexual or parthenogenetic species can eliminate gene flow even more effectively than ploidy changes alone. If sperm is not required, the newly derived clonal species instantaneously begins an existence independent of the progenitor. The asexual daughter species of *Cnemidophorus* no longer needs males to produce, however, reproduction in females of the asexual species are facilitated by a curious male-like reproductive behavior exhibited by females. If females participate in pseudocopulation in which one female mounts another a female in the stereotypical male-female copulatory position, the female lays more eggs, and the interclutch interval is reduced (Crews 1983).

Interestingly, the block to the formation of hybrids is not necessarily absolute in all parthenogenetic species and genes from a facultative asexual species can be introgressed into sexual species. Most of the time the asexual species produce progeny by parthenogenesis. For example, asexual species of flatworms from lakes in Europe have evolved repeatedly (Michaels personal communication). Many flatworms are simultaneous hermaphrodites (see Chapter 8), which maintain active testes and ovaries. While the testes of the parthenogenetic species of flatworms are much reduced, they still produce viable sperm. The asexual species of flatworm can produce haploid sperm that it can transfer back to the sexual species, which allows for one way gene flow from the facultatively asexual daughter species, back into the parental sexual species.

Multiple-Niche Polymorphism in Insects and Host-Plant Preference

Without strong assortative mating of some kind sympatric speciation is an impossibility. Any sympatric speciation theory that involves a gradual change in sympatry is difficult because gene flow would swamp the build up genetic differentiation that forms the hallmark of speciation. There is perhaps a notable exception, which involves the evolution of

insect preferences for feeding on certain species plants. It is thought that the following mechanism may be why there are more insect species of insects on the planet than any other organism. If so, then this mechanism is truly important from the point of view of animal behavior and diversity. Part of the reason why it is so prevalent is because insects can rapidly evolve specialized detoxifying mechanisms towards a given host plant via a specific enzyme -- P450 (the details of which need not concern us). However, the acquisition of such specificity should lead to rapid acquisition of host plant preferences -- a behavioral trait.

Many host plants of insects produce very nasty chemicals in response to being eaten. The chemicals are meant to deter the herbivores. In order to continue feeding on the plants, insects must evolve mutations that allow them to detoxify the chemicals. Because different host species produce very different toxins, the mutations that allow an insect to detoxify one host-plant's arsenal of toxins do not work with another plant.

Thus, one class of speciation models deals with evolution of **multiple-niche polymorphisms**. Heterozygotes are inferior because extreme specialization in detoxifying capability is necessary to successfully survive and reproduce on different species of plants. Assortative mating among homozygotes is a key component of this theory. This minimally requires two loci to evolve simultaneously, and thus strong linkage disequilibrium must build between the niche polymorphism locus and the assortative mating locus. More loci make it unlikely to occur.

An insect must evolve the detoxifying mutations, and also evolve host-plant preferences that allow it to become very choosy about the kind of plants upon which it (or its larva) feeds. The mutations do not have to occur simultaneously, but the detoxifying mutation probably arises first and the behavioral preference evolves as a refinement to the detoxifying phenotype. Individuals that match their own detoxifying abilities with the proper host plant choice would be strongly favored by natural selection. This can arise readily via correlational selection (Chapter 3).

In general, two such mutations are required, because it is difficult to imagine getting a single mutation that has a pleiotropic effect on detoxifying ability and host-plant preference. The two mutations should also arise on the same chromosome in close proximity to one another. This causes the behavior for host-plant specificity to be tightly correlated with the detoxifying genes. The linkage between the two loci

forms a genetic correlation between the behavioral and physiological traits. An insect that ends up with the wrong allele for detoxifying matched up with the wrong preference is in trouble (recall the problem for striped and spotted snakes). By having the mutations on the same chromosome, a super gene for detoxification and host-plant preference is produced. If the two loci are unlinked at the outset, it is possible for the genome to become rearranged after the two mutations arise. However, this would require a third mutation, a chromosomal rearrangement, which makes it less likely (Felsenstein 1981).

The incipient insect species, which feeds on a new host plant species is found in the same area as the ancestral species, but by virtue of different host plant preferences and mating microhabitats, they no longer exchange genes. The easiest way for many insects to find suitable hosts for their own progeny would be to oviposit their eggs on the host plant. If you oviposit on your host plant, chances are mating would also occur on the plant prior to oviposition. This speciation process not only leads to adaptive plant preference on the part of the insect, but because other members of the insect's own incipient species also choose the same plants, the insects will mate with like members of their incipient species and not with other members, and reproductive isolation is achieved.

Sympatric versus Allopatric Speciation

Assortative Mating is required for Sympatric Speciation

The formation of new sexual species by changes in ploidy and the formation of asexual species by parthenogenesis are both examples in which the new species becomes reproductively isolated from the parent. How likely is sympatric speciation in other groups if there is any gene flow between incipient species. Lets re-visit an old system -- the beak morphs of African seed crackers (Smith) and speculate about what might happen to *Pyrenestes* in the not too distant future. If S (small-billed) birds are much more successful in feeding on small seeds and L (large-billed) birds are much more successful in feeding on large seeds, why shouldn't these two morphs begin to form a new species, each specializing on a specific species of sedge? Natural selection on the two morphs appears to promote character divergence. Such selection on

ecological traits provides a key route for the rapid accumulation of genetic differences. Why haven't the morphs of seed crackers speciated?

Based on the calculations of observed and expected proportions of matings between small and large-beaked morphs, we have already seen that seed crackers mate randomly (see [Side Box 2.4](#)). To even have a remote chance of speciation, seed crackers would have to evolve some level of assortative mating. Without assortative mating the gene flow between the finch morphs would continue unabated.

Table 5.1. Observed frequency of large and small-beaked morphs is random (A) which contrasts strongly with patterns expected under strong assortative mating (B).

Observed	Female of the Pair		
	S	L	
Male of the Pair	S	34	14
	L	14	6

Expected assortative mating	Female of the Pair		
	S	L	
Male of the Pair	S	48	0
	L	0	48

Cultural Inheritance of Song and Speciation in Darwin's Finches

The natural selection observed in seed-cracking African Finches may form a model for how natural selection has acted on feeding behavior and performance of Darwin's Finches on the *Galápagos* Islands. For Darwin, the *Galápagos* Islands shaped his thinking like no other place on the planet. These islands continue to be a source of inspiration for field biologists interested in the evolution of behavior. Peter and Rosemary Grant have carried out a series of elegant studies that document the role of species song in maintaining species differences.

Each of the twelve species of Darwin's Finches have evolved specialized morphologies and behaviors that are adapted to their ecological niche (Grant, 1986). On some islands one finds two or three species with drastically different beak morphologies that they use in feeding. One

species of finch has even evolved a woodpecker lifestyle in that it feeds on insects under bark. Interestingly, this species does not use its bill to extract the insects, rather it has evolved tool-using behaviors. The species picks up twigs that it uses to probe the nooks and crannies of bark to extract insects. The males of each species also have unique species-specific songs, and females strongly prefer song types of their own species to other species (Grant and Grant 1996a,b,c).

Most of the larger islands in the *Galápagos* archipelago have several species of finches, which raises questions regarding the speciation events that have produced the diversity of finch species. Have the species of *Galápagos* finches evolved by sympatry on the same island? Alternatively, have the species evolved by founder effect speciation or speciation in allopatry on adjacent islands. To get more than one species on each island requires subsequent re-invasion events between islands. At least one species must disperse to another island where a second species is found. The two species begin competing on some of the same resources, however, by a process of **ecological character displacement** on feeding morphology (Grant, 1986) each species evolves specialized behaviors and morphology that allow the species to specialize on certain resources such as plants with different sized seeds. Thereafter **reproductive character displacement** may cause divergence in their song due to hybrid unfitness of hybrids with intermediate morphologies.

The evolution of new types of *Galápagos* finches is due a major gene called *Bmp4* that causes changes in beak development (Abzhanov et al. 2004). Each morph feeds on a slightly different resource, much like the morphs of seed-cracker in Africa. However, matings between morphs of seed crackers from Africa produce perfectly viable young, and these morphs are considered a single species. In addition there are no hybrids in seed crackers. There are heterozygotes, but they remain hidden by the dominant effect of the large beak allele. Finally the lack of assortative mating of morphs of African finches suggest that speciation is unlikely from ecological polymorphism *per se*. Thus, sympatric speciation based on the model of of seed cracker morphs from Africa, may not provide a complete explanation of speciation among *Galápagos* Finches.

Available data on the success of hybrids between different species of *Galápagos* finches suggests that hybrids have high viability (Grant and Grant 1996). Moreover, the hybrids are intermediate in terms of many

morphological traits, such as beak shape. One of the key requirements for sympatric speciation would be a *lower fitness of hybrids* that would then favor the evolution of *mate discrimination* mechanisms between the two sympatric species of *Galápagos* finches. Where hybrids are formed between species, the hybrids appear to quite viable. In the absence of such a fitness cost, it is unlikely that species recognition mechanisms evolved in sympatry, which is also consistent from a study of *Galápagos* finch phylogeny (Stern and Grant, 1996).

Evolution of species in allopatry is a more likely mechanism in light of the lack of hybrid inviability. In allopatry, species would acquire differences in ecology, as well as important differences in song. When the dispersal of birds occurs among islands, the long distances between islands minimize gene flow. On each island, the incipient species would begin to diverge from their parental species. Upon re-invasion and establishment of sympatry, evolved differences in song type among males, and female choice would tend to maintain differences among species. In order to understand speciation

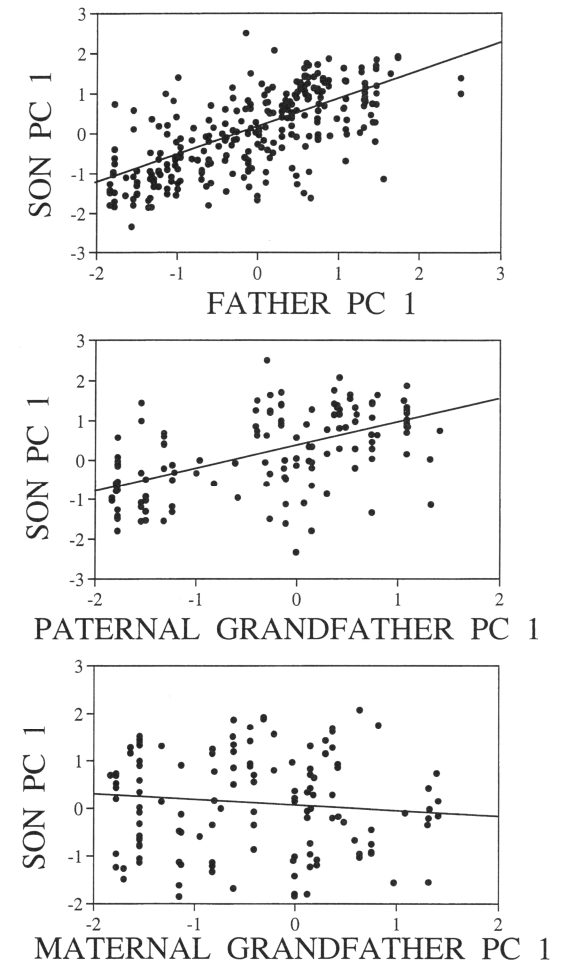


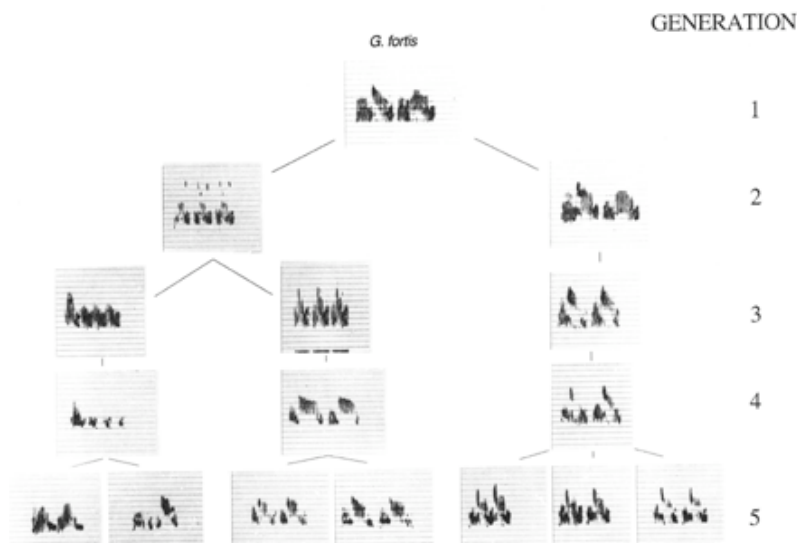
Figure. 5.16 Similarity of song from sire to son, and paternal and maternal grandfathers to son in *Geospiza fortis*. (Grant and Grant 1996).

mechanisms in Darwin's Finches we need to understand the transmission of song between generations, which is the sexually selected trait. In passerine birds or songbirds, this occurs by **culture** rather than genes.

Darwin's finches transmit song from father to son. Moreover, the transmission of song from father to son is likely to be cultural and not genetic. If song is genetic, we would expect that a male bird could transmit genes for song to their daughter. While daughters do not sing their grandfathers song, they would still be expected to pass the genes for song on to their own progeny. Consequently, there should still be a positive correlation between maternal grandfather's song and the mother's son. However, there is no relationship *G. fortis* (Fig. 5.16). In contrast there is a strong correlation between the paternal grandfather's song and son, and a stronger correlation between father and son. This indicates that song has a patrilineal inheritance, and moreover, song is culturally transmitted.

The work of the Grants on the Galápagos Islands suggests that the transmission of dialect occurs via paternal lineages by the process of cultural evolution. Mistakes are occasional made in the transmission of song from father to son. In such cases the progeny may learn a song

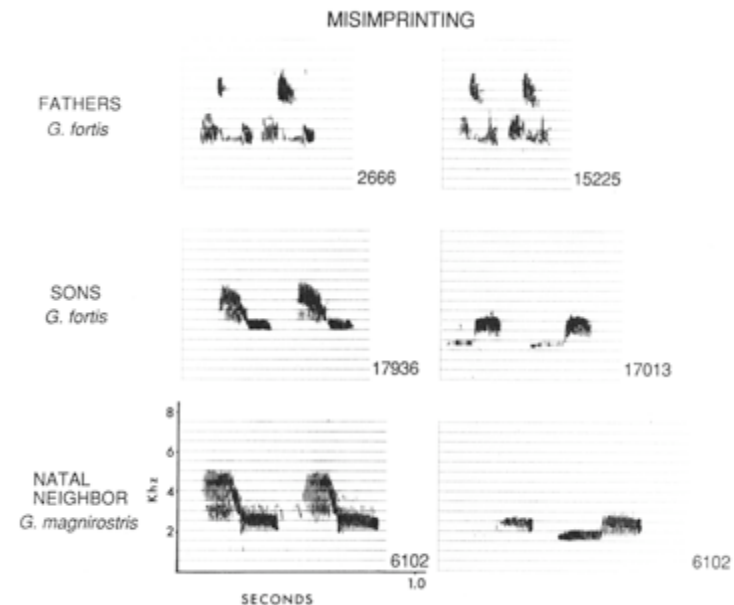
Figure 5.17. Transmission of songs from father to son in *Geospiza fortis* across five generations. While most males faithfully copy their father's song (right hand lineage), some copy the song of another male (see left lineage).



from a neighboring male that is of a different species and such misimprinting might increase the likelihood of the misimprinted male of one species pairing with a female of the wrong species.

The cultural transmission of song has several important consequences for speciation mechanisms in Darwin's Finches (Grant and Grant, 1996). First, cultural transmission of song allows for misimprinting to occur (Fig. 5.17-18). Sons learn a song during a critical developmental period (see chapter 17, development and environment). If the father dies prior to this imprinting period, a neighbor may fill in the role of song tutor. The son learns the neighbor's song. In some cases, the juvenile birds learn the song of another neighbor that is a different species (see Fig. 5.18). While rare, such cases of misimprinting allow for genes to flow from one species of sympatric Darwin's finch to another. Finally, while females of Darwin's finch do not learn to sing a father's song, they learn to recognize the song of their father and prefer to mate with males that sing the species specific song that they learned during the nestling phase. Most of the time, Darwin's finch correctly learn song as males, and females and thus correctly choose males as mates based on song.

Figure 5.18. Misimprinting of song in *Geospiza fortis* sons that had *Geospiza magnirostris* neighbors (Grant and Grant 1996).



Deme Recognition Female Choice for Natal Song

An additional requirement on speciation in songbirds is for the female to evolve, such that they prefer a song from their own deme compared to adjacent deme. A complete answer to this question requires an experimental approach to the female song recognition process, which owing to the sensitive nature of Galápagos Islands, is impossible to perform on Darwin's Finches. Females in many bird species appear to be sexually responsive to mates from their own **natal areas**. For example white-crowned sparrows show tremendous variation in dialect among populations (Marler and Tamura, 1964) separated by only a few km. The following experiment illustrates that tutors transmit songs to nestling females and then females mate on the basis of this learned recognition.

Baker (1981) used tutor males to teach young female birds a song dialect. He found that female white-crowned sparrows were more receptive to male courtship songs if the song was derived from their natal dialect with which they were tutored compared to an alien dialect (Fig. 5.19). The response used to determine receptivity was **lordosis** in which the female elevates her tail in preparation for copulation.

A researcher can tutor females into preferring a truly "non-natal" song with an alien dialect. Playing them a song with an alien dialect during the critical song-learning period in nestlings causes a switch in

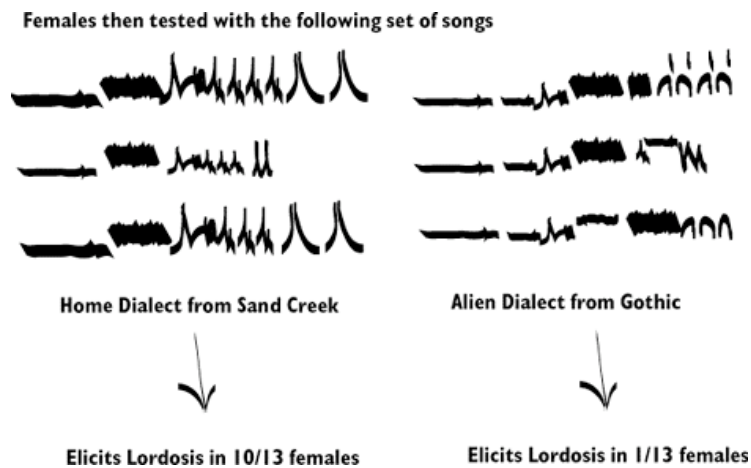


Figure 5.19. Selection of songs by males used to tutor females.

preference of females at maturity. For example, tutoring nestling females with an alien Gothic dialect caused mature females to prefer Gothic dialects at maturity over their natal dialect, the Sand Creek song.

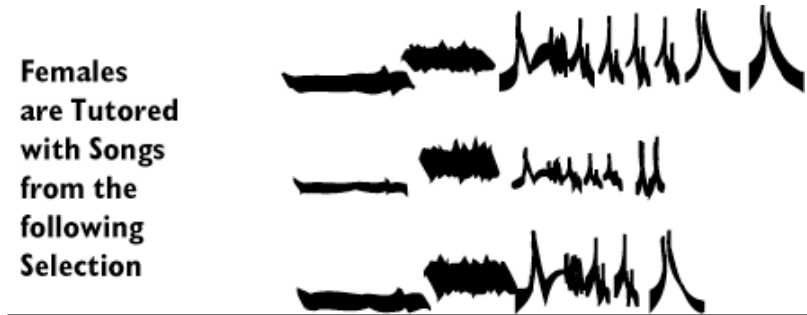


Figure 5.20. Sonogram with an alien dialect (Gothic top) and natal dialect (Sand Creek) were presented to nestling and mature Sand Creek females.

This study demonstrates that female birds learn the song during the nestling phase. Species recognition mechanisms in songbirds rely on the cultural transmission of song in both sexes. However, results from Grants' study suggests that allopatry may be required to develop the initial differences in song between incipient species.

Sympatric speciation in viduine finches and host song

There are cases where sympatric speciation is possible in some songbirds. Brood parasites lay eggs in nests of conspecifics or other species, to be cared for by hosts. To reduce reproductive costs of brood parasitism (Payne et al. 2001), hosts evolve defenses including refined recognition of parasitic eggs and chicks, nest defense (Amundsen et al. 2002), or egg counting (Lyon 2003). Brood parasites, counterselected to combat host defenses, evolve refined egg mimicry (Soler et al. 2003) and signals to enhance host feeding (Lyon et al. 1994). Coevolved strategies of hosts and brood parasites are thus coupled by very strong frequency dependent selection (Soler et al. 2003). Viduine finches provide a dramatic example of **mimetic** evolution and speciation. Indigo birds, *Vidua chalybeata*, learn songs of host species, and as adults, males attract females with songs of foster parents (Payne & Payne 1994). Mutual production and preference of mimetic host songs in both sexes reflect sexually and parasitically selected traits. Imprinting of female brood parasites as chicks on their hosts song attracts them to mates that sing the song of the same host. Imprinting also attracts them back to

nests of specific hosts in a culturally selected runaway (Payne et al. 2000). Positive FDS is held in check by negative FDS and counterstrategies by hosts that limit a specific memetic lineage of Viduine finches. The process of culturally transmitted song in both host and parasite has generated a high speciation rate in the Viduine finches.

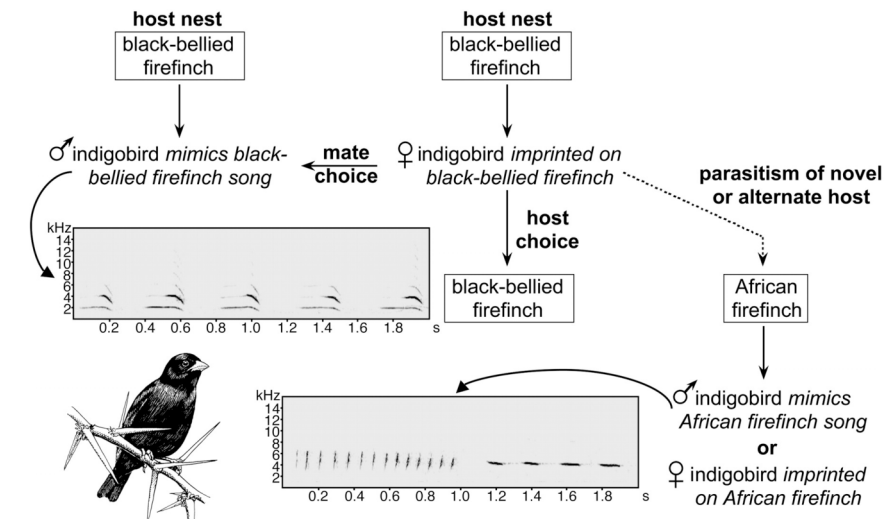


Figure 5.21. Schematic of cultural transmission of song in both males and females that is used in mate choice, as well as in the female's ability to find its host. This two-part use of the host's song (mate choice, host finding) in the parasite fuels very strong positive FDS that reflects a culturally selected runaway since success of female's in host finding is directly related to preference of females for the song that the male can sing with high fidelity.

Reinforcement and Sympatric Speciation

The example of Darwin's Finches suggest that acquisition of differences in song in allopatry can lead to allopatric speciation, which are propagated by cultural inheritance once the species come back into contact. Are there any convincing cases where behaviors have played a major role in the formation of species in sympatry? New evidence suggests that male plumage and female choice may have facilitated sympatric speciation between two species of flycatchers (Sætre et al 1997). Male collared flycatchers, *Ficedula albicollis*, have a bright

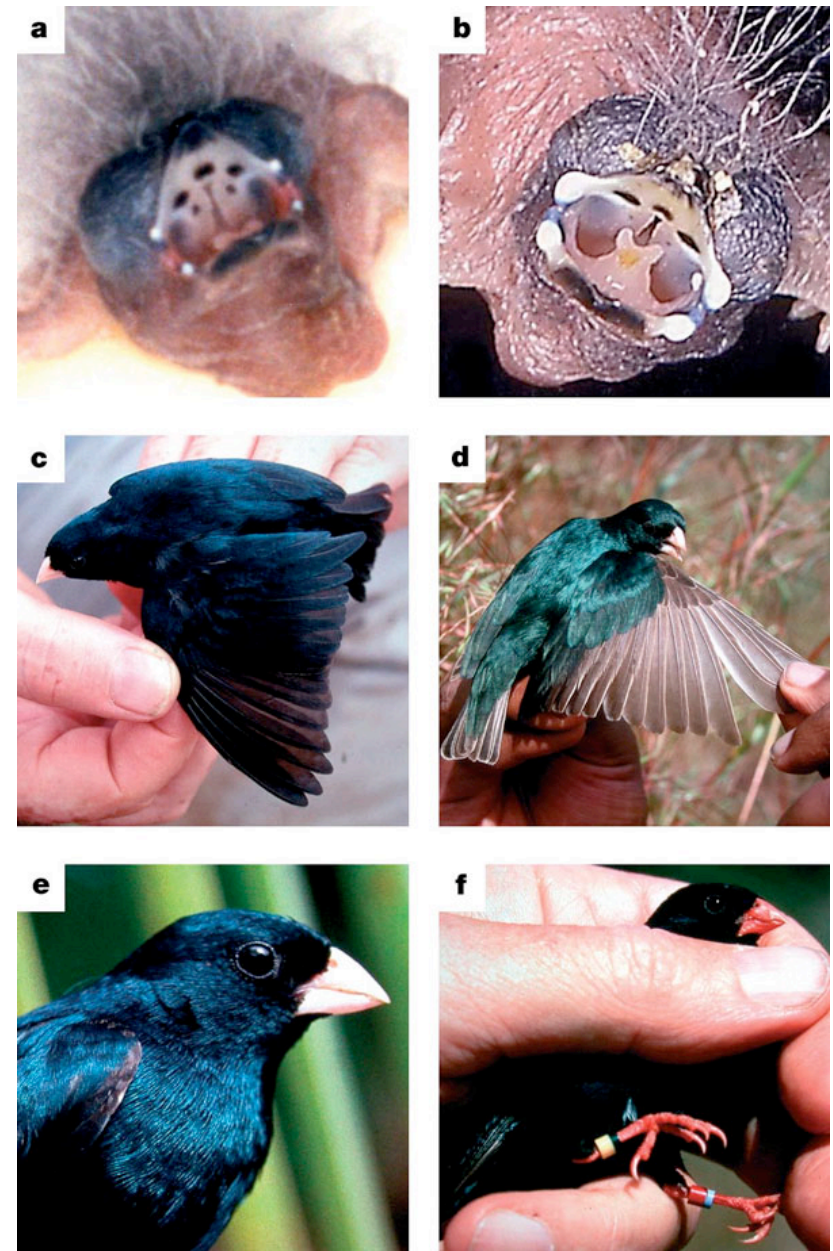
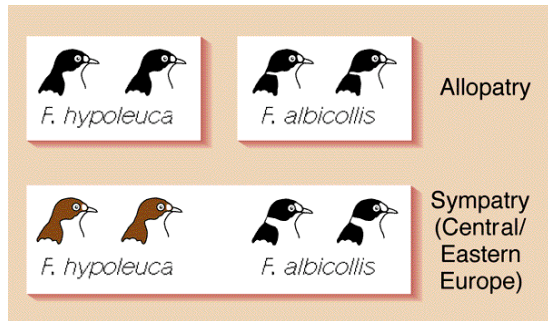


Figure 5.22. Mimicry in progeny begging morphology between an indigo bird and its host (host a), parasite (b).



white collar that contrasts with dark body plumage. The closely related pied flycatcher, *F. hypoleuca*, also has white and dark plumage where it is found in allopatry. However, in areas of sympatry, male

collared flycatcher have evolved an even more enhanced collar. In sympatry, male pied flycatchers have evolved drab plumage. The evolution of drab coloration in the pied flycatcher is particularly striking because bright white and black plumage is a strongly sexually selected trait in allopatric parts of the species range (Sætre et al 1995). Selection for reproductive character displacement in pied flycatchers may have overwhelmed the force of sexual selection for bright contrasting plumage that is typical in allopatry, creating drab brown plumage.

Each species of flycatcher has diverged in male signal in areas of sympatry compared to areas of allopatry. Females have also evolved much stronger mating preferences in areas of sympatry. In particular, pied flycatcher females strongly prefer drab male flycatchers compared to the more ancestral bright white plumage coloration (Fig. 5.23). Male traits and female preferences have diverged in areas of sympatry relative to allopatry (Fig. 5.24). The underlying cost of hybridization, which causes a pattern of sympatric speciation and reinforcement like this, must also be present (Butlin, 1987). Whereas pure and mixed species pairs have a low hatching failure rate of 4.9%, pairs with a single hybrid had a 74% hatching failure rate. Therefore, there is a severe reproductive cost to pairing with the wrong type, but it is realized in the F2 generation. While mixed pairs do not pay a current cost for disassortative mating the cost is paid when their F1 hybrid progeny mature and experience high rates of hatching failure in F2 progeny. All of the crucial aspects of reinforcement theory appear to be present in the case of sympatric speciation between pied and collared flycatchers. The birds exhibit more refined mate choice in the area of sympatry. Males have diverged in their signals in the area of sympatry, which helps females make the right choice during the reproductive season. Hybrid unfitness drives speciation and reinforcement.

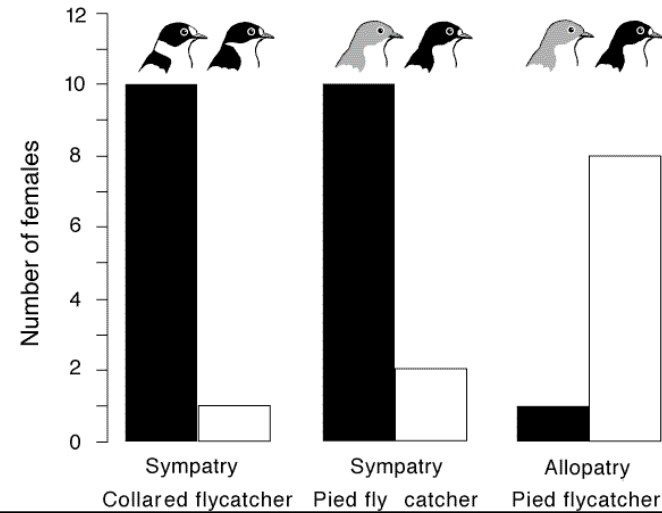


Figure 5.23. Mate preferences of female flycatchers. Left, female collared flycatchers preferred male conspecifics with large patches of white to those with reduced patch. Centre, sympatric female pied flycatchers preferred brown colored male conspecifics to black-and-white ones. Right, allopatric female pied flycatchers preferred black-and-white colored male conspecifics to brown ones. Mate preferences of sympatric female pied flycatchers were opposite to those of allopatric ones. (Sætre et al. 1997).

Figure. 5.24. Species recognition by female pied and collared flycatchers in the area of sympatry. Species assortative mating was significantly reduced when males of the two species had plumage characteristics typical of allopatric populations (small difference observed in the histograms on the right) compared to when males had plumage characteristics typical of sympatric birds (large difference in the histograms on the left). (Sætre et al. 1997).

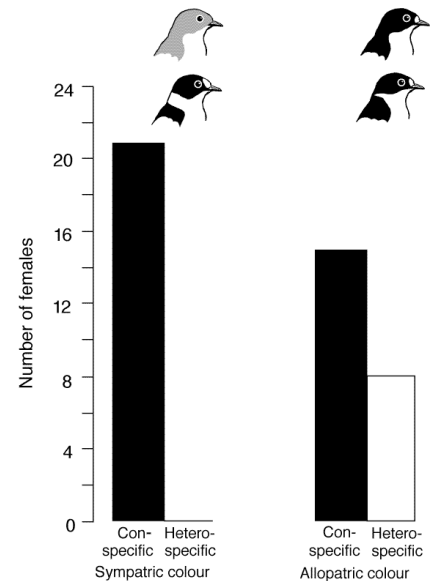
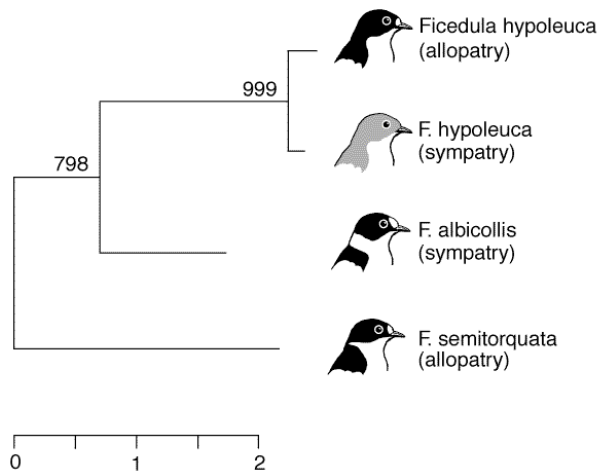


Figure 5.25. Phylogenetic relationships among sympatric and allopatric flycatchers based on mitochondrial DNA sequences. The phylogenetic reconstruction suggests that the plumage characters in sympatry are derived traits, supporting the argument of a sympatric character divergence.



The last critical piece of evidence is an important phylogenetic consideration. The actual trait(s) in the zone of sympatry should have arisen after the species entered the zone of sympatry such that the inference can be made that the displaced trait is a direct response to contact with a form that is similar. This also appears to be the case. *Ficedula hypoleuca* in sympatry with brown color is recently derived from an allopatric population of *F. hypoleuca* that retains the black color, but lacks the collar. Likewise the derived condition in sympatric *F. albicollis* of a wider collar should be derived from a more ancestral condition of a narrow collar for *F. albicollis* in the area of allopatry. The ancestor *F. semitorquata* has a narrow collar (Figure 5.25). Therefore, the evolved brown condition in sympatry appears to be due to reinforcement *per se*, and not merely some spurious association of male traits that is due to an ancient split between the two species.

Experimental demonstration of reinforcement

Compelling examples of reinforcement are beginning to accumulate and even the theory (Servidio and Saetre 2003) is catching up to the empirical evidence. Reinforcement can occur under the right conditions (e.g., those listed above). However, a compelling experiment is often

required to prove an idea that is as controversial as sympatric speciation (Rice and Hostert 1991). An experimental test of laboratory evolution suggests that reinforcement is quite likely. This example not only highlights the experimental approach, but it introduces an additional sensory mode to our growing repertoire of animal behaviors, olfaction. Many insects use cuticular hydrocarbons to signal and the example of the fire ant greenbeard in Chapter 4 is thought to arise from a cuticular compound of some sort. Species of *Drosophila* use many signaling modalities in mate choice and speciation including wing displays, sound produced by the male's wings, as well as cuticular hydrocarbons.

Higgie et al. (2000) characterized a contact zone between species of Australian *Drosophila*. Larger differences in the composition of cuticular hydrocarbons (CHC) are observed between the two species in sympatry compared to allopatry (Figure 5.23).

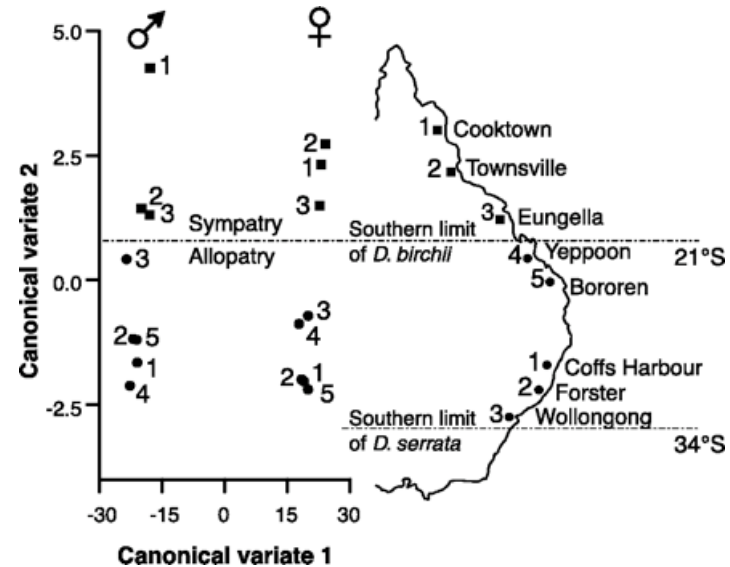
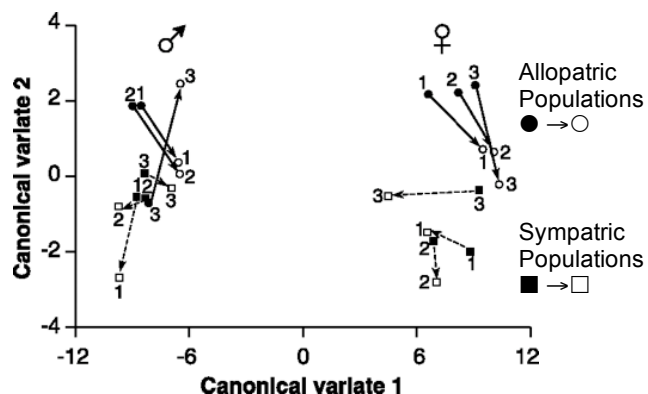


Figure 5.26. Reproductive character displacement in the cuticular hydrocarbons of *D. serrata*. The map of eastern Australia shows the distributions of *D. serrata* and *D. birchii* as well as the positions of three sympatric and allopatric populations of *D. serrata*. Population means are from the first two canonical variates from a canonical discriminant analysis conducted using the CHC data from the control populations from the selection experiment plus the two additional populations (4 and 5). The CHCs of *D. serrata* exhibit reproductive character displacement, changing abruptly at the sympatry-allopatry border. From Higgie et al. 2000.

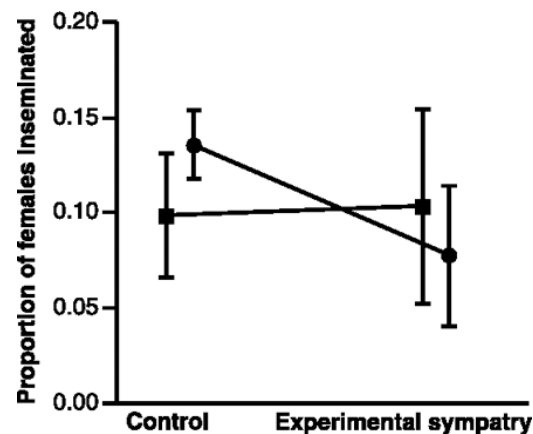
Higgie et al. (2000) took individuals from *D. serrata* populations located in the zone of allopatry into the laboratory and subjected them to a laboratory environment where they had to discriminate mates from amongst breeding *D. birchii*. After 9 generations allopatric *D. serrata* evolved large differences in CHC profiles (Fig. 5.27) in the direction observed in naturally occurring sympatric *D. serrata* populations.

Figure 5.27. The effect of natural selection on cuticular hydrocarbons of *D. serrata* after 9 generations of experimental sympatry with *D. birchii*. Evolutionary responses are indicated from control populations (closed symbols) to experimental sympatry populations (open symbols) of field allopatric populations (circles connected by solid arrow) and field sympatric populations (squares connected by dashed arrow). Numbers refer to localities given in Fig. 5.26. (from Higgie et al. 2000).



Moreover, the *D. serrata* males from natural sympatry and allopatry were then challenged to find and mate with females in the context of experimental sympatry in the lab. The challenge was again conducted in the presence of *D. birchii*. Field allopatric males inseminated significantly fewer females in experimental sympatry than in the allopatric controls, with nearly 50% fewer *D. serrata* females inseminated in the presence of *D. birchii* (sloped line, Fig. 5.28). In contrast, the number of females inseminated by field sympatric males was unaffected by experimental sympatry (flat line, Fig. 5.28). Selection on mate recognition therefore operated during courtship, rather than after the production of hybrid individuals with low fitness, to generate the reproductive character displacement in the CHC profile.

Figure 5.28. The effect of experimental sympatry on the efficiency of field sympatric and allopatric *D. serrata* males in inseminating *D. serrata* females. Means and 95% confidence intervals are based on the three field allopatric (●) and sympatric (■) populations. (from Higgie et al. 2000).



Speciation from hybridization

The kind of hybridization that generates polyploid species like the Hyliid frogs discussed above is relatively common. Speciation by the formation of a **homoploid hybrid species**, in which ploidy level does not change, is however, extremely rare. Until now, the only convincing example is for a sunflower (Rieseberg et al. 1996). In homoploid hybrid species, the genomes of two parental species hybridize to create a new type. Different genes from each parent type become fixed for one parental species or the other. Many cases of homoploid hybrid speciation are suspected in plants, which can hybridize between extremely different species to generate a hybrid in which a block to further backcrosses with parental species can arise rapidly. Few cases are suspected in animals.

I cannot resist writing about a final example, which involves *Heliconius* butterflies. I believe that homoploid hybrid speciation might be quite common in nature since animal species hybridize all the time. However detecting its action requires a lot of genomic evidence. One has to show that a species is formed from the union of two other species genomes,

which requires marker loci across the genome of three species. One must also show that blocks to mating arise rapidly. This is only likely if a behavioral block arises, which is only likely in animals if pre-zygote mating behavior evolves rapidly or instantaneously with the formation of a new homoploid hybrid species. Selection must be strong if the block is not instantaneous. Selection in the case of mimicry is very strong.

Heliconius melpomene (red on a black background) and *H. cydno cordula* (white-yellow band on a black background), have evolved differences in mate choice in the area of sympatry however, they do occasionally form hybrids. As noted above, F1 female hybrids are sterile, however, F1 male hybrids are fertile and can be backcrossed to either parental type, thereby recovering fertile F2 females that have various morphologies, spanning the range of variation between parental species (Fig. 5.29). Color patterns are controlled by 3 co-dominant loci. *Heliconius heurippa* has an intermediate wing pattern, which has led to the suggestion that it is a hybrid. Its hindwing is indistinguishable from that of sympatric *H. m. melpomene*, whereas the yellow band on its forewing is similar to that of parapatric *H. cydno cordula*.

A random selection from F2 hybrids, however, never breeds true because of the large number of combinations among 3 loci (Fig. 5.29). Mavárez et al. (2006) selected *heurippa*-like types from F2 hybrids and crossed this with other *heurippa*-like types. This type does breed true because the *heurippa*-like type in the F2 progeny array is homozygous at all 3 loci that control forewing and hindwing color. Therefore the likely route to homoploid hybrid speciation is via an F2 hybrid, which evolved in parapatry from its parental species. The additional assortative mating within type must have arisen in parapatry. In addition, Mavárez et al. (2006) suggest that ongoing frequency dependent selection by the predator, which favors a *heurippa*-like type and disfavors other F2 hybrid combinations, would also have had to be present. As we will see in the chapter on predator learning (Chapter 14), this possibility is likely.

Mavárez et al. (2006) do not identify specific loci of mate choice that evolved in the *heurippa*-like ancestor, but *H. heurippa* prefers to mate with self-types like its parental species *H. melpomene* and *H. cydno*. Indeed, while the genes for signals have been mapped or sequenced in many the examples listed above, the actual genes that control mate choice remain elusive in all of these animal systems.

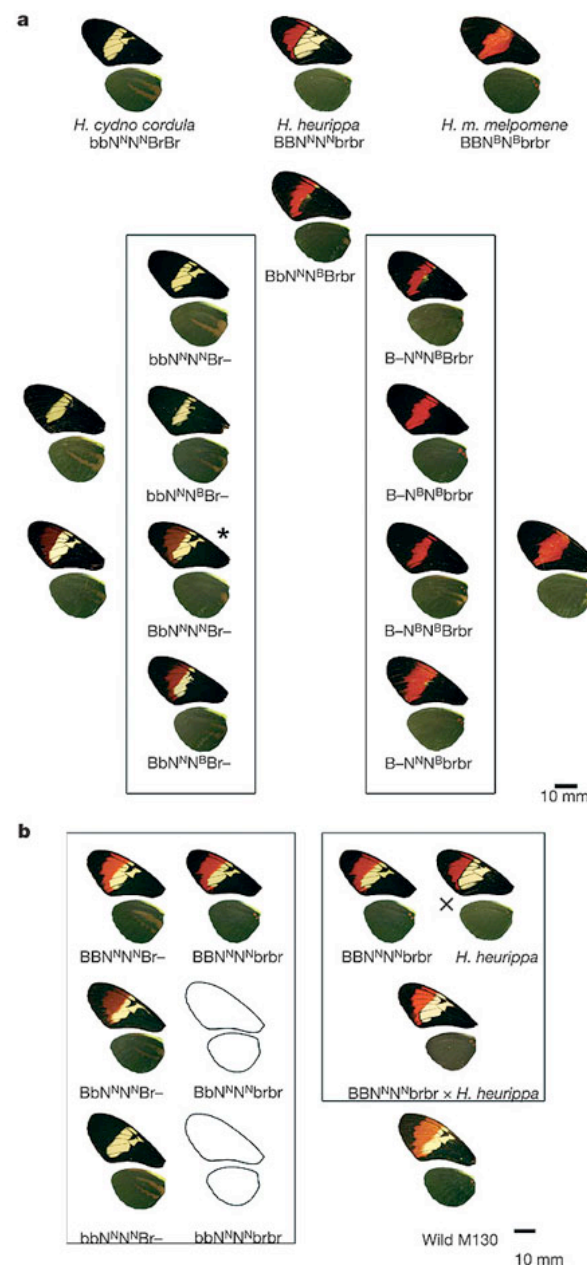


Figure 5.29. Reconstruction of the *H. heurippa* wing pattern. All fore- and hindwings are in dorsal and ventral views, respectively. (a) **First row:** *H. cydno cordula*, *H. heurippa* and *H. m. melpomene*; **second row:** *H. cydno cordula* × *H. m. melpomene* F1 hybrid. Backcrosses to *H. cydno cordula* & *H. m. melpomene* are shown in the left and right boxes. Three loci regulate color patterns: **locus 1) red dorsal forewing band:** complete (BB, *H. melpomene*), intermediate (heterozygote Bb), or none (bb, *H. cydno*); **locus 2) yellow band on dorsal forewing:** complete (NNNN, *H. cydno*), intermediate (heterozygote NNNB) or none (NBNB, *H. melpomene*); **locus 3) brown pincer-shaped mark on ventral hindwing:** complete (BrBr, *H. cydno*), intermediate (heterozygote Brbr) or none (brbr, *H. melpomene*). Cross for recovering *heurippa* was made within the phenotype marked with an *. (b) Left box: offspring from crosses for individuals marked with an * in (a). The B and Br loci are linked, which explains the absence of two recombinant genotypes ($BbNNNNbrbr$ and $bbNNNNbrbr$). Right box: offspring of a cross between a lab hybrid with genotype $BBNNNNbrbr$ × *H. heurippa*, showing that the pattern breeds true. M130 is a *heurippa*-like wild hybrid from San Cristo' bal, Venezuela.

Founder Effect Speciation

Founder effect speciation refers to a mechanism very similar to allopatric models of speciation. The primary difference relates to the size of the founding population, which colonizes some region on the very edge of a species range. If this founding colony remains isolated for a long period of time then speciation might occur (Mayr 1942). A key component of founder effect speciation relates to the genetic material that is brought in by the initial colonists. Because the number of colonists is very low, the sample of genetic material is much reduced compared to the source population. For example, if a gravid *heurippa*-like form spread to an isolated area, it would be homozygous for a *heurippa* pattern and its descendants would be likely to breed true. In this case, the founding population is expected to diverge from the source population from the outset. Genetic drift facilitates divergence over the long term. Many cases of island species are thought to arise by founder effect speciation. In particular the 700 species of Hawaiian *Drosophila* are thought to have arisen by founder processes in which small isolated islands of forest form after lava flows cut off these forests from adjacent populations. In isolation, the *Drosophila* are thought to rapidly evolve mate discrimination mechanisms, but the force of sexual selection and runaway process is thought to be required as well.

Summary: Runaway Process, Sexual Selection, and Speciation

The final model of speciation that we will consider is runaway sexual selection. Recall Fisherian runaway process. The essence of the theory is that a mutation for female choosiness arises in a population and the females opt for males with some kind of elaborate trait. If Fisherian runaway process is to play a role in speciation mechanisms, then it must be related to the process by which reproductive isolation occurs. How do the female discrimination traits such as that seen in ancestral flycatchers evolve? What about plumage variation seen in other bird species? How is it that species of lizards have evolved such markedly divergent push-up behavior? The theory for runaway process has an interesting second phase that may be instrumental in promoting speciation (Lande 1981).

In the first phase (Chapter 3) we saw that Fisherian runaway will lead to ever increasing escalation in the male trait up to the point where the survival costs of the trait balance sexual selection. At this point, the second phase of Fisherian runaway sexual selection takes hold.

The genes underlying the male and female traits may begin to change in frequency by the process genetic drift. **Genetic drift** is simply the random processes that cause small fluctuations in the frequency of an allele simply because of limited population size. By chance alone, the frequency of alleles (for choosiness and the male trait) will fluctuate up and down in frequency. In small populations the effect of genetic drift is expected to be quite great compared to large populations. How small is small? Generally populations that are less than about 100 individuals. What might happen is that certain alleles for choosiness might go to fixation in a small population, or perhaps genes for the male trait.

Up to this point we have considered runaway selection and its operation on a single male trait and a single mutation for choosiness. However, as discussed above sexual selection operates on the [whole organism](#) and females might choose on the basis of many male traits, which are coded for by many loci. Birds might choose both tail length as well as plumage coloration. Butterflies might choose hindwing and forewing coloration.

Imagine a number of distinct sub-populations, which are experiencing genetic drift and going to fixation for different alleles. Because genetic drift is an entirely random process we would expect that each sub-population would fix for different choosiness and along with it different male traits. Another scenario for such random effects is that the mutations, which lead to choosiness or the male trait, might differ in each sub-population. Genetic drift in combination with other random forces such as mutation can lead to local geographic differentiation.

Fisherian runaway selection might lead to rapid evolution in each population according to such initial differences. When this occurs, strong assortative mating and reproductive isolation has evolved even before the populations come into secondary contact at some future time. Populations would be expected to diverge rapidly in their mating preferences under the selective force of runaway and the stochastic process of genetic drift. The end result of runaway is reproductive isolation by mating preferences -- the key ingredient for speciation. While runaway and genetic drift is a tantalizing theoretical possibility with regards to the role of sexual selection in promoting speciation, convincing cases of its operation are not very well characterized. I have carried out a comprehensive review of studies that document assortative mating, hybrid unfitness, and those that include phylogenetic inference.

Moreover, theoretical models of sympatric speciation in which hybrid unfitness is included (Servidio and Saetre 2003) indicate that runaway sexual selection takes hold in each species as it forms. Processes of speciation described above, therefore occur by the same fundamental processes found in single isolated populations. The example of collared and pied flycatchers indicates that sexual selection favoring highly contrasting plumage can be overwhelmed by the force of reinforcement speciation that favors drab male pied flycatchers in the area of sympatry.

The key feature that is different in speciation models, compared to simple models of runaway sexual selection is that the hybrid unfitness poses an adaptive challenge for females and males. They must co-evolve both preferences that discriminate species and signals that distinguish mates from other species. In chapter 10, we will explore more complex models of sexual selection that show females within a population are likely to choose males based on signals that enhance progeny quality. In this regard the mate choice that evolves at contact zones enhances the fitness of progeny because low fitness hybrid progeny are formed at a lower rate. This interesting process of sexual selection just happens to be the powerful engine of new biodiversity on the planet – speciation.

Whether or not a given mode of speciation (e.g., allopatric versus sympatric versus reinforcement) is responsible, critically requires female preferences and male traits to evolve in concert with the factors that promote hybrid unfitness. In addition, detailed information on phylogeny is required to reconstruct the historical pattern of each species to determine whether they are due to some allopatric process (e.g., two ancient refugia), selection in an area of secondary contact with reinforcement, or true sympatric speciation. For example, the ring species hypothesis was invalidated for species of sea gulls, which have two ancient refugia, but the ring species hypothesis was validated for warblers, which exhibit isolation by distance along the ring and strong differentiation only at the ring terminus. Phylogenetic inference was also used in the cases of mimicry (Batesian salamanders and Müllerian butterflies), and in the case of sympatric speciation of flycatchers.

Despite these historical inferences, only experiments can prove the action of speciation processes. This is why experiments like those on experimental sympatry (*Drosophila*) are critical. Experiments allow us to determine the plausibility of speciation under controlled conditions.

Study Questions for Chapter 5

1. What are the two critical conditions for sympatric speciation?
2. Why do behavioral isolation mechanisms between species evolve? Isn't sterility enough to keep species from mixing?
3. How does the evolution of host-plant choice satisfy the conditions required for sympatric speciation?
4. Describe an experimental design, which manipulates species recognition cues by altering the signals that are important for recognition.
5. When the two ends of a ring species meet, individuals do not interbreed and they evolve a behavioral isolating mechanisms (e.g., *Ensatina* salamanders), how can they remain separate species if gene flow is possible all along the length of the ring?
6. Which mode of selection may play a major role in speciation and the evolution of species-recognition mechanism. (an answer requiring information from chapter 3).
7. Describe a female choice experiment that tests for the presence of reproductive character displacement. Assume that you can make synthetic male signals or displays.
8. Describe a lab challenge that tests for the evolution of reproductive character displacement in experimental sympatry.
9. Can culturally transmitted traits generate speciation?