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Recombination and the divergence of hybridizing species

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Abstract

The interplay between hybridization and recombination can have a dramatic effect on the likelihood of speciation or persistence of incompletely isolated species. Many models have suggested recombination can oppose speciation, and several recent empirical investigations suggest that reductions in recombination between various components of reproductive isolation and/or adaptation can allow species to persist in the presence of gene flow. In this article, we discuss these ideas in relation to speciation models, phylogenetic analyses, and species concepts. In particular, we revisit genetic architectures and population mechanisms that create genetic correlations and facilitate divergence in the face of gene flow. Linkage among genes contributing to adaptation or reproductive isolation due to chromosomal rearrangements as well as pleiotropy or proximity of loci can greatly increase the odds of species divergence or persistence. Finally, we recommend recombination to be a focus of inquiry when studying the origins of biological diversity.

Introduction

Theoretical models have suggested that recombination can oppose species formation or the persistence of hybridizing species in numerous ways (see Table 1). For example, in a classic theoretical model of hybridizing subpopulations, Felsenstein (1981) showed that linkage disequilibrium between loci conferring adaptation to different environments and a locus for assortative mating substantially favors the divergence of these subpopulations through fixation of alternate alleles. If recombination breaks the allelic association between mate choice and adaptation, speciation does not proceed. Empiricists also noted the ideas of species acting as 'coadapted gene complexes' (Mayr, 1963), 'fields for recombination' (Carson, 1975), or bearing 'supergenes' of tightly linked loci that confer fitness advantages in specific environments (e.g., Anderson et al., 1975; Turner, 1967a,b). In any of these cases, recombination between different types could break apart these complexes, resulting in the formation of unfit progeny.

In recent times, several authors have presented empirical evidence of reductions in recombination or evolved genetic correlations possibly contributing to species formation or persistence (e.g., Hawthorne & Via, 2001; Noor et al., 2001c; Rieseberg, 2001). Interestingly, although these many authors, early and recent, have reached similar conclusions, their rationales differ on *why* such genetic associations may aid the speciation process. In this article, we review the empirical results suggesting the importance of genetic associations on speciation, and we review some similar suggestions from theoretical studies of related disciplines (e.g., sexual selection).

The fundamental association between recombination and speciation is rarely noted. If species are considered to be entities capable of exchanging genes, or populations within which adaptive variants can spread, then the complete absence of recombination (defined in this article as the mixing of genetic material

Table 1. Studies examining the effect of recombination between adaptation and/or reproductive isolation components on the course of divergence

Study	Geography	Fitness or reproductive isolation components	Effect of free recombination
Barton and Turelli (1991), Kirkpatrick (1982), Lande (1981)	Sympatry	Female preference and sexual male trait	Approach to equilibrium can be retarded
Barton and Bengtsson (1986)	Hybrid zone	Viability selection	Reduces the strength of the barrier to gene flow
Felsenstein (1981), Barton and Hewitt (1985)	Sympatry, hybrid zone	Premating and postmating	Stable polymorphism, divergence less likely
Hostert (1997)	Laboratory sympatry	Premating and postmating	No reinforcement
Kirkpatrick and Barton (1997)	Sympatry	Coadapted traits	Decreases indirect selection
Li et al. (1997)	Laboratory hybridization	Fitness	Destruction of supergenes
Liou and Price (1994)	Hybrid zone	Female preference and sexual male trait	Decreases divergence when there is significant hybrid fitness
Sanderson (1989)	Cline	Fitness component and fitness modifier	Modifier that is favored in both races is hindered by recombination
Servedio (2000)	Hybrid zone	Female preference and sexual male trait	Reinforcement less likely in two-island model
Tricket and Butlin (1994)	Sympatry	Premating and postmating, female preference and sexual male trait	Divergence less likely

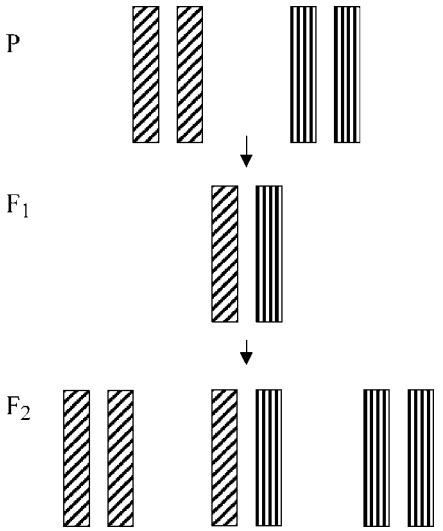


Figure 1. Two non-recombining genomes are not able to mix their genetic material since an F_1 individual can only produce parental types or offspring identical to itself after crossing with another F_1 or backcrossing.

due to either independent assortment of chromosomes or crossing over within chromosomes) in hybrids between two taxa would by definition cause speciation. Two completely non-recombining genomes could come together in a heterozygous form, but introgression could not occur from one taxon into another because of the absence of any form of recombination (see Figure 1). Any adaptation within one taxon could not spread into the other, as would be true of two species that produce completely sterile hybrids. This extreme example illustrates how recombination is intrinsically tied to speciation in the population genetic sense.

Means by which genetic associations can arise

Recombination can be reduced among loci in many ways, thus producing genetic correlations among alleles or phenotypes. Crossing over is reduced between

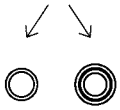
	Sympatric	Allo-sympatric	Allopatric
 Ancestral population	Disruptive selection (ecological, sexual, antagonistic pleiotropy)	Physical barrier	Physical barrier
 Split	Differential adaptation and/or runaway sexual selection – continuous gene flow may be present during sympatric speciation	Accumulation of adaptive differences and genomic incompatibilities	Accumulation of adaptive differences and genomic incompatibilities
 Hybridization	Prezygotic isolation (assortative mating, runaway selection)	Reinforcement	N/A
 Speciation	Spread of adaptive variants is confined to each species	Spread of adaptive variants is confined to each species	Spread of adaptive variants is confined to each species

Figure 2. Some modes of speciation. Adaptive variants within a population may be confined to subpopulations due to the action or natural selection (sympatric speciation) or presence of physical barriers (allo-sympatric and allopatric speciation). Adaptation occurs, gene flow decreases with time incompatibilities accumulate exponentially. Eventually, gene flow is not possible and new species are formed.

physically proximate loci or loci in centromeric (e.g., Lindsley & Sandler, 1977; Nachman & Churchill, 1996; Payseur & Nachman, 2000) or telomeric regions (e.g., Carpenter, 1979). Similarly, chromosomal rearrangements (e.g., inversions) may effectively impede crossing over along the rearranged region in heterozygotes through the lack of recovery of recombinant progeny. Genomic rearrangements are widely known and reported from natural populations of numerous species (e.g., Shaw, Wilkinson & Moran, 1983; Anderson et al., 1991; Wallace & Searle, 1994; Li et al., 1997; Rieseberg, Whitton & Gardner, 1999). However, evolutionary processes such as runaway sexual selection, genetic drift, admixture, or nonadditive fitness interactions among loci may also produce genetic correlations among loci independent of genome organization. During runaway sexual selection, a cyclic coevolution of two alleles with sex-specific effects produces a genetic correlation between female preferences and preferred male characters (Fisher, 1930; Lande, 1981; Kirkpatrick, 1982), whereas genetic drift and admixture produce non-random associations among alleles at many loci by reducing the sample of possible genotypes (e.g., Li & Nei, 1974; Pritchard & Przeworski, 2001).

The processes that create genetic correlations may facilitate speciation by allowing combinations of alleles among genes contributing to adaptation or reproductive isolation to persist in hybridizing taxa. To understand their effects on the evolution of reproductive isolation, we will focus on cases of sympatric speciation and secondary contact after speciation has begun in allopatry ('allo-sympatric'), since the concept of hybridizing taxa is applicable to either case despite the difference in when gene flow occurs (Figure 2).

In this article, we classify the mechanisms that create genetic correlations among loci as structural and population-based mechanisms (Table 2). In the presence of a structural mechanism, recombination is reduced due to genome organization. Structural mechanisms may involve a single gene that has pleiotropic effects on the phenotype, or more than one locus

Table 2. Some means by which genetic correlations can arise

1. Structural
A.Linkage due to pleiotropy
B. Linkage due to proximity
C. Linkage due to chromosomal rearrangements
i. Additive model
ii. Negative epistatic model
2. Populational
A. Sexual selection
B. Genetic drift
3. Allopatry

contributing to a phenotype. Population-based mechanisms include sexual selection, genetic drift, or recent admixture. Below, we discuss many of these mechanisms with respect to speciation, with emphasis on structural mechanisms and discuss some recent studies that exemplify the interplay between recombination and components of reproductive isolation.

Genetic associations due to allopatry

Although this mode of speciation does not involve gene flow, allopatry is the simplest means by which nonrandom associations among alleles may appear. After populations cease to exchange migrants (see Figure 2), new gene variants are fully restricted from recombining between populations. Thus, there will be complete linkage disequilibrium among fixed differences between populations and proportionally lower linkage disequilibrium between polymorphisms that are at different frequencies. If populations were to come into contact again, recombination would be restored and genetic associations would dissipate with time. Below, we discuss the means by which genetic associations may persist in the presence of gene flow.

Pleiotropy or linkage due to proximity

Speciation is facilitated when loci conferring traits undergoing disruptive selection, such as by specialization to different hosts, are physically linked to or identical to loci conferring mating discrimination (e.g., Felsenstein, 1981; Rice & Salt, 1990). Because recombination would be suppressed between these loci or effects, the genetic correlation among alleles of particular types would persist longer in the face of hybridization. In the course of habitat specialization, habitat preference should become associated with habitat performance, as organisms should tend to choose habitats in which they will have the highest fitness (e.g., Diehl & Bush, 1989; Via, 1990; Thompson, 1994; Berlocher & Feder, 2002). In addition, as offspring develop on the hosts their parents preferred, they would undoubtedly be subjected to selection pressures of performance on that host, and host performance and preference will become even more associated. Sympatric speciation may then accompany this habitat divergence if mating only occurs in the preferred habitat, hence associating mate preference with habitat preference. This linkage of habitat performance, habitat preference, and assortative mating may contribute to the frequent proposed sympatric divergence of phytophagous insects.

To observe the predicted pattern of linkage associated with sympatric divergence, there must be genotype-based habitat preference differences within species or between diverging species (e.g., Taylor & Powell, 1978), and this preference should be associated with host performance. For example, patterns consistent with genetic linkage of host performance and assortative mating have been observed in closely related pea aphids that specialize on different hosts (Hawthorne & Via, 2001). In these aphids, host performance is positively correlated with contypic mate choice, and discriminant individuals typically exhibit poor fitness on hosts used by the other type. Interestingly, quantitative trait loci for host performance and assortative mating (through habitat choice) map to the same genomic regions, suggesting, albeit not proving, physical linkage between loci. This genetic architecture would potentially increase individual fitness and would tend to spread easily in the population, perhaps completing the speciation process. Hawthorne and Via (2001) suggest that this type of genetic architecture may be common in taxa that have speciated under divergent natural selection, as is speculated for other phytophagous insect species (Singer, Ng & Thomas, 1988).

In a variety of other organisms, however, the genetic relationships between host performance and preference are not as clear as suggested by Hawthorne and Via (2001). In some cases, genes affecting for example larval performance on hosts are unlinked to those affecting adult oviposition preferences. This lack of association has been observed in brown planthoppers (Sezer & Butlin, 1998) and swallowtail butterflies (Scriber, 1986; Thompson, Wehling & Podolsky, 1990; Nitao et al., 1991; Scriber, Giebink & Snider, 1991): in the latter case, genes affecting oviposition preference but not those for host-related performance map to the sex chromosome.

Similarly, the relationship between host performance and preference is absent when studying an adaptive trait in the species *Drosophila simulans* and *D. sechellia*. These species are largely allopatric, though they now co-exist on one island of the Seychelles, likely due to a recent secondary contact. *D. sechellia* has specialized on and prefers to oviposit on the fruit of *Morinda citrifolia*. This fruit is toxic to *D. simulans* larvae, and *D. simulans* females actively avoid ovipositing on it. In contrast to the situation above, the limited genetic data available suggest that the oviposition preference for and adaptation to morinda fruit are not linked in *D. sechellia*: the region of the genome with

the strongest effect on oviposition preference is on the 2nd chromosome (Higa & Fuyama, 1993), while this chromosome has only a very weak effect on resistance (Jones, 1998). As these species probably have come into contact only recently, no linkage is expected under the model above, and the data provide a good contrast to the results from studies of sympatric pea aphids.

In summary, we can conclude that the evolutionary relationship between host performance and preference is somewhat unclear, but the little genetic evidence for its existence suggests that it is sometimes associated with a genetic architecture that favors divergence in sympatry. This genome architecture is characterized either by pleiotropy or close linkage between genes for host performance and preference. Hence, recombination does not impede divergence.

Linkage due to chromosomal rearrangements

Chromosomal rearrangements, such as chromosomal inversions or translocations, may allow factors conferring adaptation or reproductive isolation to be genetically correlated when not physically proximate along chromosome arms. The effect of such rearrangements would be analogous to physical linkage or pleiotropy, as offspring of heterozygotes would possess the entire rearranged region from one or the other taxon. If these rearranged regions bear alleles under divergent selection or conferring reproductive isolation, rearrangements could potentially suppress the opposing effect of recombination to sympatric speciation or reinforcement described in the theoretical models referenced in Table 1. One might predict that, in general, most homosequential regions of the genome would tend to introgress more easily between hybridizing species than rearranged regions because of incomplete linkage to alleles conferring adaptation, mating discrimination, or hybrid dysfunction. Rearranged regions may sometimes be completely linked to such alleles, and introgression will be more limited.

These expectations have been demonstrated in empirical studies. For example, rates of gene flow are higher between homosequential than between rearranged chromosomes in sunflower hybrid zones (Rieseberg, Whitton & Gardner, 1999). Rieseberg, Whitton and Gardner (1999) examined three hybrid zones of two sunflower species inhabiting Nebraska. Assuming that homosequential regions of the genome would introgress between species, Rieseberg, Whitton and Gardner (1999) were able to estimate the deviation from expected numbers of introgressed markers occurring in these hybrid populations. Remarkably, they found that most markers in homosequential regions of the genome tended to be observed at neutral expected frequencies, whereas markers from regions bearing inversion or translocation differences between the species were almost always underrepresented in the hybrid zone. These rearranged segments were commonly associated with pollen sterility in crosses between parental population, demonstrating greater divergence in isolating factors in rearranged than in homosequential regions.

Similar results were obtained from genetic studies of hybrid sterility, sexual isolation, and other barriers to gene exchange between the hybridizing species *D. pseudoobscura* and *D. persimilis*: all effects mapped primarily or exclusively to regions bearing fixed inversion differences between the species (Noor et al., 2001b, c). Sequence analyses also suggest that these inverted regions do not introgress between these two species as well as homosequential regions (Wang, Wakeley & Hey, 1997; Machado et al., 2002). These results suggest that

(a) gene pools are semi-permeable,
(b) chromosomal rearrangements may prevent gene flow across large genomic regions, and
(c) chromosomal rearrangements may play an important role in the genetic isolation of species in the presence of hybridization.

Two recent non-mutually-exclusive explanations have been proposed whereby chromosomal rearrangements, such as inversions, may facilitate the persistence of hybridizing species, hence explaining the results described above. We call these the ‘additive model’ and the ‘negative epistatic model’ for simplicity. Each is described in turn.

Additive model. The additive model (Rieseberg, 2001) suggests that chromosomal rearrangements prevent gene flow between hybridizing species by summing the effects of genes conferring adaptation or hybrid dysfunction across large regions of the genome. Similarly, multiple adaptive or isolating alleles within inversions would reduce further the possibility of gene flow for all loci in the inverted regions. Recombination is effectively suppressed across rearranged regions in heterozygotes (hybrids), the fitness effects of the alleles at the individual loci are summed, and the entire region behaves as a single allele possibly under very strong selection. Hence, when hybridizing taxa differ in gene arrangement, and the rearranged regions

contain multiple genes conferring adaptation or reproductive isolation, then gene flow can be substantially reduced or prevented across a large fraction of the genome.

Negative epistatic model. Noor et al. (2001c) have forwarded another explanation for why chromosomal inversions may aid the speciation process. This model focuses on hybrid dysfunctions in particular, and it rests on two additional assumptions, each of which has been supported through empirical data. First, the genetic incompatibilities that produce hybrid sterility are typically asymmetric: an allele from species A will produce hybrid sterility in the genetic background of species B, but the alternate allele at the same locus from species B will not necessarily produce hybrid sterility in the genetic background of species A (see Johnson, 2000). This assumption may be supported by the work on *Odysseus*, which produces sterility when introgressed from *D. mauritiana* into *D. simulans* (Perez et al., 1993), but several introgressions in the other direction are fertile (Palopoli & Wu, 1994). It is also supported by the frequent observation of F_1 hybrid male sterility in one hybridization but not the reciprocal. Second, the model assumes that many loci possess alleles that can confer hybrid male sterility, consistent with high-resolution genetic data from the *D. simulans*/*D. mauritiana* group (Wu & Hollocher, 1998).

Noor et al. (2001c) suggested that large chromosomal rearrangements produce a symmetric hybrid male sterility effect from asymmetric genetic incompatibilities. After hybridization, recombination can eventually tie together alleles from the two species not conferring hybrid sterility onto the same chromosome in homosequential regions. This recombinant chromosome will be fully fertile, and introgression can occur. In contrast, when hybrids inherit entire rearranged regions from one species or the other, either of these regions will often possess alleles that confer sterility in the foreign genetic background. Because recombination does not occur, each arrangement will continue to be associated with hybrid male sterility in the foreign genetic background in succeeding generations. In classical genetics terms, the alleles from each species that do not cause sterility are trapped in repulsion phase and cannot come together into coupling phase. Hence, reduced recombination via rearrangements prevents introgression of these regions into the foreign species. This process therefore allows the hybridizing species to persist, as complete fusion cannot occur.

This model works best if hybrid sterility is caused by negative epistatic interactions between loci on two or more chromosomes rearranged between the hybridizing taxa. For example, in *D. pseudoobscura* and *D. persimilis*, the strongest negative interactions that cause sterility occur between loci on the inverted XL and the inverted second chromosomes. Had only one of these chromosomes been inverted relative to the other species, then the loci with which the remaining inverted region interacted may have recombined to eliminate the sterility phenotype, and fusion would still occur.

The strength of barriers to gene flow

Now let us suppose recombination is restored after secondary contact. During the initial formation of the hybrid zone, recombination will be restored and introgression will vary with the number of genes that contribute to fitness reduction in hybrids. Genes may contribute to fitness reduction because they are incompatible in heterospecific genetic backgrounds or because they have undergone adaptive divergence in the previously isolated populations. The rate at which neutral markers will introgress into a heterospecific background is a function of the recombination rate in the chromosomal region where this marker resides, the deleterious effects to which neutral markers are linked, and their distance along the chromosome from the selected genes (Barton & Bengtsson, 1986). This can be mathematically expressed as $L_{10} = v_i \alpha r$, where L is a matrix representing the number of genes that will go from population 1 into population 0 (effective migration rate); v_i is the number of neutral markers in a chromosome carrying i deleterious effects, and αr is the probability that the neutral marker will recombine away from the deleterious effects. This computation is done over all possible hybrid backgrounds and is subsequently used to generate a mathematical recursion describing the barrier strength to gene flow generated by n selected loci.

Barton and Bengtsson's (1986) general results are consistent with the verbal arguments discussed in this article and add to our understanding of the process of speciation in several ways. First, their results demonstrate that the relationship between recombination and selection intensity greatly affect the maintenance and strength of barriers to gene flow. Second, recombination will be prevented across much of the genome if multiple genes contribute to hybrid fitness reduction, so neutral markers are more likely to be linked

to barriers to gene flow. As a consequence, early speciation events are characterized by differential introgression between hybridizing populations. Finally, we can infer from the results of Barton and Bengtsson (1986) that chromosomal rearrangement bearing genes reducing hybrid fitness are likely to present a powerful barrier to gene flow since any neutral marker contained within the rearrangement will be less likely to recombine away from alleles conferring deleterious effects.

Dynamic mechanisms

Correlations evolve between preferences and fitness traits or between preferences and preferred characters in various sexual selection models. These correlations can sometimes be favored by reduced recombination among traits. The effect of recombination has been studied primarily in the context of runaway sexual selection. Runaway sexual selection results in the joint evolution of a preference and a preferred trait in the absence of direct viability or fertility selection on the preference locus (Fisher, 1930; Lande, 1981; Kirkpatrick, 1982). Lande (1981) showed that female preferences evolve as a correlated response to selection on males. This process occurs through a genetic correlation between the loci independent of their linkage relationships, and this disequilibrium is maintained by selection. However, limited recombination can accelerate the approach to equilibria in some sexual selection models, though it may not affect the final equilibrium condition (Kirkpatrick, 1982; Barton & Turelli, 1991). Similarly, one study noted that having a lower recombination rate between female preference genes and male trait genes can enhance the effectiveness of runaway sexual selection (Otto, 1991). This is especially true if a rare allele affecting female preferences arises within a population in which a male trait is maintained as an overdominant polymorphism. The rare preference allele will easily spread in the population by association with heterozygotes at the male trait locus if recombination is low between the loci (Otto, 1991). Reducing recombination between coadapted fitness alleles may also increase the force of indirect selection on female preference (Kirkpatrick & Barton, 1997), and decreased recombination among male trait and female preference loci may increase the likelihood of speciation by runaway sexual selection (Trickett & Butlin, 1994; Takimoto, Higashi & Yamamura, 2000).

Linkage or associations of loci contributing to preferences and different fitness components may also have an impact on the dynamics of 'good genes' sexual selection. A variety of sexual selection studies have suggested that offspring of attractive males have high fitness through enhanced growth, fecundity, viability, or attractiveness (Hamilton & Zuk, 1982; Welch, Semlitsch & Gerhardt, 1998; Roulin et al., 2000; Kotiaho, Simmons & Tomkins, 2001). In these situations, preferred male characters are positively correlated with fitness, and the associations among these loci are generally thought to enhance the progress of sexual divergence and speciation. As with runaway sexual selection, recombination can retard the evolution of this correlation, so if these characters are initially positively associated, as by linkage, sexual selection will be more efficient.

However, recombination does not necessarily retard the progress of sexual selection. The generally positive effect of reduced recombination on sexual selection may be more applicable to the allo-sympatric scenario depicted in Figure 2 than sympatric divergence. In the allo-sympatric case, the initial disequilibrium between alleles that must remain together will generally be positive, thus facilitating persistence in the face of gene flow (e.g., Kirkpatrick & Ravigné, 2002). However, when divergence begins in sympatry, the initial disequilibrium between alleles that must spread together may be positive or negative. If these alleles are in repulsion phase, then reduced recombination may initially impede or prevent the progress of sexual selection.

Also, in contrast to many sexual selection studies, Brooks (2000) found a *negative* correlation between male attractiveness and offspring survival to maturity in guppies. Brooks noted that genes for ornamentation have been mapped to the nonrecombining Y-chromosome of guppies, which would place them in tight linkage with several genes that affect fitness. Sexual selection may be efficient at spreading Y-linked preferred male characters because all male offspring inherit the preferred trait. However, because much of the Y-chromosome is nonrecombining, deleterious Y-linked alleles cannot be shed by recombination, so deleterious alleles can accumulate at other loci and hitchhike via this sexual selection. Hence, in these guppies, the benefit of mating with attractive males is opposed by reduced offspring survival following such matings and sexual selection is impeded by the absence of recombination. This circumstance may be fairly unusual for most sexual species, though, as it

relies on a large, almost-completely-nonrecombining region bearing the loci that confer preferred male characters.

Effects of recombination on evolutionary studies

Speciation is the process by which recombination between genomes of subpopulations is minimized through time due to strict allopatry, accumulation of genomic incompatibilities, or adaptive divergence. It is generally accepted to have occurred when two gene pools can come into contact and yet remain distinct from each other. This process of divergence is sometimes, but not always, gradual, and it may involve phases in which introgression occurs in some parts of the genome between the divergent populations. The porosity of this process has important implications for our understanding of modes of speciation because the unit of study becomes those portions of the genome that fail to recombine between diverging taxa.

Similar ideas have been put forward through the years (Carson, 1975; Barton & Hewitt, 1985; Harrison, 1993), albeit without the genetic data now available. For example, Carson (1975) suggested that the diploid chromosomal system provides a 'field for genetic recombination' where only a portion of the field is amenable to exchange between species. The remaining chromosomal fraction would consist of balanced blocks of genes under strong natural selection precluded from recombining with another species since unfit offspring would be produced. Thus, in hybridizing species, introgression would occur only outside the balanced blocks. Although Carson's concept is consistent with our description of the events immediately following hybridization, it has conceptual differences from our suggestion of how species originate. While we suggest that recombination is reduced through time by accumulation of genomic incompatibilities between hybridizing species or adaptive divergence, Carson implies that the reduction in recombination is a fixed measure that defines the species itself. Under Carson's analogy the reduction in recombination is never completed and gene flow eventually obliterates incipient species. As a result, Carson suggests species are likely to originate through a genomic disorganization mediated by bottlenecks that shift the gene pool from one coadapted block of genes to another block, and he concludes that sympatric speciation and reinforcement are not likely to occur. Our model does not make such assumptions and is consistent with various modes of speciation with gene flow.

In retrospect, the extent of introgression between species will greatly depend on recombination rates as well as the genetic architecture of the adaptations, mate choice, or incompatibilities that have accumulated and differentiate the taxa. Incompatibilities may be distributed across large fractions of the genome or may be concentrated in few regions. Their effects may be extended or localized based on the organization of the genome in which they are located. Additionally, genetic incompatibilities may hitchhike with other alleles contributing to adaptation or mate choice if they are closely linked, are included in chromosomal rearrangements together, or co-segregate together more often than expected due to coevolution.

As described above, several studies have found that traits involved in adaptive or reproductively isolating differences between diverging taxa map preferentially to regions of the genome with reduced recombination (e.g., Feder, 1998; Rieseberg, Whitton & Gardner, 1999; Hawthorne & Via, 2001; Noor et al., 2001c). Some of this tendency may come from intrinsic biases in the way genetic mapping studies are performed (Noor, Cunningham & Larkin, 2001a), but the weight of evidence suggests that such regions may truly harbor a disproportionate number of such alleles. An interesting avenue of research would be to experimentally induce chromosomal rearrangements in two homosequential species, allow them to hybridize in the laboratory for many generations, and see if this tendency can be reproduced experimentally.

Recombination and phylogenies of closely related species

Varying rates of introgression between diverging taxa can greatly complicate phylogenetic analyses. This complication reduces (or eliminates) fixed genomic DNA sequence differences between hybridizing taxa, or it may cause sequences from some regions of the genome to suggest one phylogenetic relationship while others suggest different relationships. Recombination is intrinsically tied to these complications, as regions where recombination is effectively eliminated will yield similar phylogenies when studied. For example, if loci that cannot introgress (due to adaptation or reproductive isolation) are within regions inverted

between hybridizing taxa, the lack of introgression of these loci will be extended to all other genes in this inverted region.

An empirical example of this suggestion comes from research on the *D. pseudoobscura* group, comprised of the two subspecies *D. p. bogotana* (bog) and *D. p. pseudoobscura* (ps) and the sibling species *D. persimilis* (per). Bog and ps are estimated to have diverged approximately, 150,000 years ago (Schaeffer & Miller, 1991), while ps/bog and per diverged approximately 500,000 years ago (Aquadro et al., 1991; Wang, Wakeley & Hey, 1997). ps and per co-occur and hybridize in nature (Dobzhansky, 1973; Powell, 1983), while bog is allopatric to the other two taxa. All components of reproductive isolation map primarily or exclusively to the fixed inversion differences on the X and 2nd chromosomes (Noor et al., 2001b, c).

Recently, Machado et al. (2002; unpublished data) sequenced several loci of these three taxa and constructed phylogenies based on these sequences. Sequences of loci within the fixed inversion differences clearly distinguished ps/bog from per, as predicted from numerous other characters, while loci across most of the remainder of the genome yielded poor phylogenetic resolution. Interestingly, when mitochondrial DNA sequences were examined, ps and per appeared to be much more closely related to each other than either was to bog. As the mitochondrion is not associated with any known barriers to gene exchange in these species (Hutter & Rand, 1995; Noor, 1997), it has freely introgressed between ps and per (Powell, 1983), while other parts of the genome, and especially those within fixed inversion differences, have not. Hence, sequences from the different parts of the genome suggested dramatically different phylogenetic relationships among these taxa.

However, we need to be cautious when inferring phylogenetic relationships based on genes that putatively cannot introgress between species. Factors conferring hybrid sterility today may have evolved their sterility effect subsequent to the speciation process and may have introgressed between species earlier in evolutionary divergence. Thus, present-day incompatibilities may not necessarily reflect the speciation history of these taxa.

One possible such misinterpretation comes from a phylogenetic study of the *Odysseus* gene, which confers sterility in hybrids of *D. simulans* (sim) and *D. mauritiana* (mau). Early phylogenetic studies of these two species and their sister species, *D. sechellia* (sec), had yielded numerous potential relationships (see Kliman et al., 2000), many of which conflict with each other. Ting, Tsaur and Wu (2000) studied this triad using the *Odysseus* gene sequence, suggesting that it should not introgress between sim and mau, and should therefore present a more accurate representation of species relationships. Gene flow between sim and mau (e.g., Ballard, 2000) may have contributed to the complications in the earlier phylogenetic studies.

Ting, Tsaur and Wu (2000) found many shared-derived sites (synapomorphies) in *Odysseus* sequences that would cluster sim with mau more than either to sec, and they argue that this phylogeny more likely represents the species phylogeny since *Odysseus* cannot introgress between sim and mau. However, they fail to consider three aspects of this argument. First, if *Odysseus* confers sterility only between sim and mau, why were synapomorphies not noted in other genes? If gene flow is occurring at much of the remainder of the genome between sim and mau, and sim and mau are the ingroup species relative to sec, then sim and mau should cluster even more tightly when introgressing genes are studied than when *Odysseus* is examined. Their argument would have applied only if sim and sec clustered together, as was originally suspected (Palopoli, Davis & Wu, 1996). Second, *Odysseus* confers sterility in one genetic background: there is no consistent fitness consequence for introgression of the *D. simulans* allele into *D. mauritiana* (Palopoli & Wu, 1994), which is what is assumed to have occurred with the mitochondrial DNA sequences of these species (Ballard, 2000). Hence, unidirectional gene flow may have been possible at *Odysseus* in the recent past, and their study is not necessarily more conclusive than studies of the many other loci that also presently show fixed differences between these species (see Kliman et al., 2000). Finally, as described above, we do not know when *Odysseus* acquired its hybrid sterility effect. An estimated, 120 genes may contribute to sterility in these species (Wu & Hollocher, 1998), and *Odysseus* may have been among the last to evolve its effect on hybrid fertility. The phylogenetic relationship based on *Odysseus* gene sequences is not compelling.

Recombination and species concepts

One question that remains unresolved by a focus on recombination is the question of how to identify species. If species are evolutionary entities within which gene exchange occurs and between which gene exchange does not occur, then most speciation genetic

studies are necessarily addressing properties of partial species. Thus, for example, *D. pseudoobscura* and *D. persimilis* are only partially reproductively isolated and have been exchanging genes in nature. This partial isolation permits genetic research, but the subjects of that research are, in a critical genetic sense, not complete species. Two sorts of conceptual entanglements are associated with this uncertainty. First, since we cannot easily do genetics on completely isolated taxa, we cannot fully address whether the incomplete stages that we study are representative of early stages of speciation. Second, the presence of natural gene flow between purported 'species' creates a context that is without the reproductive isolation that inspires such investigation.

The path through this particular species muddle is to see that, throughout this article and others like it, the critical focus is not on the distinction between two species, but rather the presence of barriers to recombination. It is the origin of such barriers that permits diversity to accrue between entities. We may think of those entities as species, but it is even more useful to think of them as gene complexes (Mallet, 1995). When the subject of investigation is envisioned as recombination, per se, then questions regarding the degree of distinction between entities that might engage in recombination fall by the wayside.

Consider Dobzhansky's case for the concept of a Mendelian population: a reproductive community that shares a common gene pool (Dobzhansky, 1951). Mendelian populations need not be completely distinct, and indeed have no particular necessity for distinction, and they can be nested within one another. Dobzhansky devised the idea to help biologists think more about the factors that affect gene movement, and less about whether or not particular populations warrant some systematic status. If we adopt this viewpoint, and consider the degree and circumstances of recombination to be the focus of inquiry, then we can study the origins of biological diversity without regard to question, about whether or not the organisms we study belong to one or two species.

Conclusions

Recombination can retard species formation or persistence in numerous ways that researchers are only now beginning to understand. Although theoretical studies have varied recombination rate to investigate its effects, empiricists are just beginning to examine its role in speciation and species persistence in natural systems. Its effect appears nontrivial, and its implications span many evolutionary issues, such as the genetics of sexual isolation, phylogenetics, and the nature of species. Indeed, when reduced recombination is considered, previously controversial modes of speciation such as sympatric speciation and reinforcement become more plausible. Future speciation studies should consider recombination as a fundamental variable in the process and how it can impact their findings.

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