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Population genetic keys to speciation

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Abstract — Based on the notions of Mendelian population and biological species, the commonly proposed pathways of speciation are discussed with respect to the roles played by reproductive isolation as well as pre- and postzygotic incompatibility. It is emphasized that evolutionarily meaningful analyses of speciation processes require consideration of the conditions for establishment of the various mechanisms of isolation and incompatibility, which, in turn, necessitates specification of appropriate modes of inheritance of these mechanisms. Properties desirable for genetic models of speciation are derived and compared with existing models. A general result on the joint evolution of underdominance in fitness and positive assortative mating is used to evaluate the significance of hybrid disadvantage and prezygotic incompatibility in speciation. The conclusions from this evaluation are compared with observations published in an analytical review of studies on incompatibilities among *Drosophila* species and with the results of a genetic analysis in two lacewing species presented as evidence for sympatric speciation.

Introduction

Among sexually reproducing organisms, cladogenetic speciation refers to a process that starts with a single reproductive community and leads to at least two such communities which are mutually reproductively incompatible. Herewith, the reproductive communities considered are populations, and the process of reproductive separation splits an initially single population into at least two different populations (with common descent) belonging to different biological species. Thus, populations rather than individuals are the constituents of a biological species, as was emphasized by Mayr [see e.g. Mayr 1963, p.20] and as is now generally accepted. Moreover, the concept of a biological species dictates that conspecific populations form a single population when merged while populations belonging to different species do not. The problem is yet, that the term “population” itself appears to suffer from ambiguous or circular definition. For example, it is quite common to define a population in terms of a shared gene pool, while a gene pool is conceived of as a characteristic of a population.

Particularly when studying mechanisms of cladogenetic speciation, it is therefore advisable to build on a definition of the term “population” that considers its role as the unit of evolution, which, in turn, is basically determined by the capacity of the individual members of this unit to have common descendants. In fact, irrespective of the underlying mechanisms, if the condition of having common descendants is not fulfilled, a collection of organisms cannot reasonably be conceived of as forming a single population. The following definition reflects this reasoning and avoids circularity [Gregorius 1983]:

> *A (Mendelian)* ***population*** *is a collection of generatively reproducing biological organisms existing under specified (environmental) conditions, in which each pair of individuals has the capacity and opportunity to have common descendants over the generations.*

Note that this definition also applies to pairs of individuals belonging to the same sex in a dioecious population, since two males or two females may have common descendants after two generations at the earliest. Moreover, the criterion of having common descendants can be fulfilled by pairs of individuals from different generations even if they do not exist at the same time. In practice, fulfillment of the criterion will be based on a projection of observable current reproductive compatibility relationships into the future. Proceeding from this idea of a Mendelian population, the common concept of biological species can be stated consistently in the following operational form [Gregorius 1983]:

> *Two (Mendelian) populations belong to the same* ***biological species*** *if there exist (environmental) conditions under which they form a single (Mendelian) population when merged.*

This formulation falls into the category of “cohesion concepts” of species introduced by Templeton [1989], since it emphasizes the “potential for genetic and/or demographic exchangeability” [Templeton 1989, p. 25]. On the other hand, simple negation of the formulation reveals that it also includes the essence of the “isolation concepts” [Templeton 1989], in that it employs reproductive discontinuity as a condition for attributing two populations to different species. However, since the present definition of (Mendelian) population is oriented at the potential for having common descendants rather than common ancestry, the present concept of species is the inverse of evolutionary or phylogenetic (or cladistic) concepts [see Templeton 1989 and the concluding discussion of Endler in the same book].

It becomes apparent from the above definition that one cannot conclude that a speciation process has been successfully completed unless conditions enabling hybridization between the populations are realized naturally or experimentally. The significance of hybridization experiments for the distinction between species was of course emphasized earlier, although, without explicit reference to a particular species concept and without stating the indispensability of such experiments or observations [see e.g. the review of Templeton 1981]. Yet, the existence of conditions under which occasional or regular hybridisation between two populations occurs is not sufficient for stating conspecificity; the formation of a single Mendelian population requires the potential for common descendants for *each* pair of members. This marks one of the central problems in studies of hybrid zones [see e.g. Hewitt 1989].

Another aspect revealed by the above definition of biological species has considerable bearing on the evaluation of the conditions for speciation provided by spatial, geographical, temporal, or ecological (including adaptive) isolation. Such isolation may imply *reproductive* isolation which, in turn, provides the conditions for populations to evolve independently of each other. The latter is generally accepted to facilitate the evolution of reproductive incompatibility between conspecific populations. In the particular context of adaptive divergence of gene pools, Templeton emphasizes in his review [1981, p.27] the possibility that reproductive incompatibility may "arise as a pleiotropic consequence of this divergence". Despite the intuitive appeal of this hypothesis, the author apparently found little unambiguously corroborating experimental evidence. This is probably not surprising if one considers that, according to the above definition, the reproductive incompatibility characterizing different biological species can only show up in the absence of extrinsic forces causing reproductive isolation.

In contrast to the hypothesis of independent evolution, one could therefore argue that reproductive incompatibility can only evolve *within* a population but might result in incompatibility between reproductively isolated populations. The reasoning could be that, if reproductive incompatibility of one type of individual with other types of individuals confers any advantage at all to the first, this can only be selected for if both types are in reproductive contact, in which case the evolution takes place *within* a population. Thus, evolution of incompatibility *between* isolated populations must be preceded by the establishment of incompatibility relationships within at least one of these populations. It is the purpose of the present paper to elaborate these ideas in connection with the pertinent pathways of speciation and to draw some elementary consequences for the design of related population genetic models of speciation.

Strictly asexual reproduction in the sense that recombination of genetic information between individuals is consistently inhibited will not be considered, since, according to the present concept, it does not allow for populations of size larger than one. Moreover, considering the possibility of conjugation among bacteria or viral recombination as an example, it is not clear as to whether indefinitely persisting asexual reproduction does or can occur at all. If it would, the impossibility of having common descendants implied that any genealogical relationship between a pair of individuals ought to be sought in the past, so that only common ancestry could define conspecificity. Yet, it is difficult to conceive of any objective criterion concerning length or number of connecting lines of descent (not mentioning degrees of genetic similarity) which determine affiliation to the same systematic category including the "species" as a special case. The same ambiguity, of course, arises with sexually reproducing organisms when evolutionary or phylogenetic species concepts are applied; common ancestry of a collection of organisms need not imply reproductive compatibility among all its currently existing parts, some of which thus might even represent different biological species (cf. e.g. the critical review of species concepts by Sluys, 1991).

Pathways of speciation

When considering modes or mechanisms of speciation, an elementary distinction must made between reproductive isolation and reproductive incompatibility, as was indicated in the last section. There is probably general agreement that the possibility of mating is the central criterion for this distinction. Based on this idea **reproductive isolation** is spoken of if extrinsic conditions exist which prohibit mating between individuals. The term **reproductive incompatibility** is generally agreed upon when addressing the situation where mating occurs but either does not result in the formation of zygotes or yields inviable or infertile offspring. Both terms, isolation and incompatibility, are used when mating is prohibited by other than external forces such as ethological or recognition barriers in animals or asynchronous flowering in plants.

In plants, differential flowering times could be caused by environmental differences (exposition, for example) only, in which case reproductive isolation would be the appropriate description, since the forces inhibiting mating are of extrinsic origin. Yet, if the flowering differences were of genetic origin, the common usage of terminology is ambiguous. To avoid such ambiguity, the term reproductive incompatibility will be extended in the present paper to include all non-extrinsic determinants of an individual that prohibit mating. Hence, genetically determined asynchrony in flowering or recognition barriers, if they lead to assortative mating, will be counted as incompatibility, while with purely environmental determination they constitute a case of isolation.

An extreme case of reproductive isolation is realized when mating is generally prevented as a consequence of incongruousness of sexual organs, for example. This case, where mating is *a priori* impossible, will be referred to as **obligatory reproductive isolation**. On the other hand, reproductive incompatibility is at issue if matings produce no offspring under some conditions, even if they do produce offspring under others. The role played by reproductive isolation and incompatibility in the classification of speciation processes will be discussed in the following.

Modes of speciation — Modes of speciation usually are distinguished according to the degree of potential reproductive contact between the populations involved. Built on a geographical conception, the terms allopatric and sympatric characterize the two extremes where populations are geographically (reproductively) isolated and where such isolation is completely absent, respectively. An intermediate form, in which allopatrically living populations exchange genes, was termed “parapatric” by Smith [1955], who also [Smith 1965] suggested “dichopatric” as a term for completely reproductively isolated allopatric populations.

Yet for the present purpose these distinctions are required only insofar as they help differentiate qualitatively between modes of speciation. In this context, the nature of the factors – whether geographic, ecologic, physiologic, ethologic, etc. – that affect the degree of reproductive isolation is irrelevant. This leaves us with two pure modes of speciation, namely speciation in the presence and in the absence of reproductive isolation among the constituent populations, which, as a consequence of more or less restricted gene flow, encompass a continuum of intermediate (parapatric) situations with respect to the degree of isolation. Apparently there is a tendency in the technical literature to address these two aspects of modes of speciation as allopatric and sympatric, respectively, even though this is not quite in accordance with the original definitions. Nevertheless, for reasons of familiarity and suggestiveness these two terms will subsequently be used in this generalized sense.

Mechanisms of speciation — There are basically two categories of forces which may interrupt the reproductive continuity realized in a Mendelian population. These categories are separated by the event of zygote formation and are accordingly referred to as pre- and postzygotically acting barriers to reproduction. The latter type of barrier results in inviability or infertility of offspring (starting at the zygotic stage) and is thus described by the term **postzygotic incompatibility** as a shortened version of postzygotic reproductive incompatibility. Similarly, the prezygotic phase of reproductive incompatibility, which, according to the above definition, comprises determinants of the formation of zygotes acting prior to and after mating but excludes factors of reproductive isolation, is termed **prezygotic incompatibility**. Hence, gametophytic systems of incompatibility in which the tubes of certain pollen types do not grow in certain styles and completely positive assortative mating (where individuals differing in type do not mate) both constitute examples of prezygotic incompatibility .

Speciation by reinforcement — In this pathway of speciation [see e.g. Dobzhansky 1941 or Grant 1966], allopatric and sympatric modes of speciation are combined with mechanisms of pre- and postzygotic incompatibility. Essentially, the idea is that during the first phase of the process, two reproductively isolated (allopatric) populations evolve (partial) postzygotic incompatibility. This does, of course, not become manifest unless secondary contact between the two populations initiates a sympatric phase of coexistence, which then gives rise to the evolution of prezygotic incompatibility. The suggestion is that the reduced fitness of the hybrids between the two populations fosters prezygotic incompatibility between these populations, thereby avoiding the production of unfit hybrids (the *Wallace effect*, after Wallace 1889, p. 175f, see Grant 1966).

As compared to the combination of allopatrically and sympatrically evolving incompatibilities alone, speciation by reinforcement appears to have attracted more interest, and in some influential publications such as that of Lewontin [1974] alternatives are not even seriously discussed. On the other hand, based on experimental evidence and model simulations, many authors hold that speciation by reinforcement as described above is hard to realize and therefore is not likely to take place under natural conditions [cf. e.g. Templeton 1981, Lambert et al. 1984, Spencer et al. 1986, Butlin 1987, 1989]. This conflict motivates reconsideration of the conditions that favour the initiation of speciation processes, which is the subject of the next section.

The initiation of speciation

Speciation is the evolution of post- and/or prezygotic incompatibility between certain characteristics for which the descendants of a formerly single population differ. It is essential to specify these (species-specific) characteristics, since there might exist other traits which do not allow for a sufficient distinction among individuals in terms of incompatibility. Hence, a decisive question is as to how pre- and/or postzygotic incompatibility can become established and can persist under allopatric or sympatric modes of speciation. For example, in a common type of model positive assortative mating is assumed to exist for the states of a specified trait, and it is demonstrated that after a sufficient number of generations and possibly supported by a suitable selection regime two trait states remain between which mating is practically inhibited (see e.g. Rice 1984). While this type of model successfully describes a final stage in the speciation process, it fails to demonstrate how positive assortative mating itself could have evolved.

In fact, any evolutionary biological reasoning explaining the existence of a certain characteristic is purely academic as long as the problem of establishment or initial increase in frequency of this characteristic is ignored. While in many cases theoretical or experimental studies of the conditions for establishment of a new phenotype may be straightforward, an analysis of the evolution of phenomena such as pre- or postzygotic incompatibility is more intricate, since the characteristics concerned refer to pairs of individuals rather than to single individuals.

This becomes particularly evident if one considers the classical model of viability selection at a single biallelic gene locus with heterozygote disadvantage (underdominance). In this model, stable coexistence of the two alleles (protected polymorphism) is impossible under random mating and random distribution of genotypes over environments. The crucial question is then, what evolutionary significance this mode of gene action in the heterozygote has, if, due to the instability, it cannot become established nor persist under these conditions. Exactly this, however, is one of the central problems emerging in the concept of speciation by reinforcement, as was repeatedly emphasized [see e.g. Templeton's review, 1981]. While genetic differentiation between reproductively isolated populations is a common phenomenon and is easily explained, for example, by adaptation to different environments or drift, the evolution of (partial) postzygotic incompatibility in the hybrids (corresponding to underdominance) is problematic. This problem arises equally with and without consideration of random variation as is evident in Wright's [1977, chapter 13] shifting balance theory of evolution and its application to theories of speciation (for a more recent discussion see Lande 1989). The following two points of view require consideration.

Postzygotic incompatibility and reinforcement — During the phase of reproductive isolation, the postzygotic incompatibility is just a non-realized potential, becoming manifest only after the isolation ceases. Hence, one is forced to explain how a particular mode of gene action can evolve in a situation where the genes involved cannot combine in a (hybrid) genotype and where this genotype thus cannot be selected. A simple explanation could be provided by assuming a difference in environment between the two populations that concurs with adaptational differentiation and leads to fixation of different alleles in the two populations at a number of loci. Even though genetically homologous, the genes (alleles), when brought together in a hybrid zygote, exhibit some sort of functional incompatibility leading to postzygotic incompatibility, the degree of which may, of course, vary between environments.

At least some of the genes or gene combinations involved in the postzygotic incompatibility reactions should not have been present in the initial population from which the two populations descend. If all genes had been present, then the "hybrid" genotypes could have been formed in the initial population, which, by definition of a Mendelian population, requires the absence of continuing reproductive isolation between the carriers of the genes concerned. This, however, would contradict the hypothesis that separation through reproductive isolation is the sole cause for the evolution of the incompatibility.

It is difficult, on the other hand, to reason why the genes present in the two populations after the isolation could not just as well have occurred (via mutation, migration etc.) in the initial population before the isolation. Hence, the conditions for establishment of these genes *after* reproductive isolation must indeed be sought in this isolation. If for at least one of the two successor populations the environmental conditions differ qualitatively from those of the ancestor population, mutants that could not have become established under the environmental conditions of the ancestor population could be successful under the new conditions (probably as a consequence of a genetic composition changed by adaptive differentiation). This situation would be relevant in the case where newly arising environments (niches) are invaded. If, however, the environments of the successor populations, though distinct from each other, are part of the environmental conditions of their ancestor population, differences in competitive conditions between the two environments can explain the establishment and fixation of previously unsuccessful mutants. This is known to occur when populations regularly living in optimal habitats (with heavy competition) colonize suboptimal (more stressful) ones (with less competition).

A second explanation for the establishment of mutants after reproductive isolation may of course be seen in mere chance events implying genetic drift after an initial bottleneck or a prolonged period of reduced population size. The differentiation in genetic compositions leading to postzygotic incompatibility between the successor populations could then result from random fixation of a mutant in one of the populations. On the other hand, the random differentiation could as well have provided the difference in genetic background required for a mutant to realize the genetic interactions allowing its adaptive establishment in one of the populations. The pros and cons of adaptive *vs.* random (stochastic) genetic differentiation are, however, not of immediate concern to the present topic and need therefore not be further discussed here.

Prezygotic incompatibility and reinforcement — While the last considerations give meaning to and provide simple explanations for the evolution of postzygotic incompatibility under reproductive isolation, the maintenance of such incompatibility after secondary contact (cessation of the isolation) until prezygotic incompatibility can reinforce the (sympatric) reproductive isolation is the second problem to be solved. The underdominance principle inherent in the hybrid disadvantage strongly suggests that for largely unrestricted mating between the populations one of the two will vanish. Hence, the danger of extinction of one of the two populations – and thus the failure of speciation – would be reduced if either (*a*) mechanisms are realized that allow the two populations to coexist despite their postzygotic incompatibility and in the absence of reproductive isolation, or if (*b*) prezygotic incompatibility would already have evolved before secondary contact (i.e. allopatrically).

In the framework of evolution by reinforcement, allopatric evolution of prezygotic incompatibility implies that postzygotic incompatibility must have evolved simultaneously ("pleiotropically", as Maynard Smith, 1966, and Templeton, 1981, put it) during the phase of reproductive isolation. The prezygotic incompatibility should then not be complete, since otherwise the existence of postzygotic incompatibility could not show up after secondary contact and would thus be irrelevant as a mechanism of speciation. Incomplete prezygotic incompatibility in turn requires complete postzygotic incompatibility to guarantee the evolution of properly separated biological species. However, the continual formation of inviable or infertile offspring constitutes a genetic load that can be avoided only by the evolution of complete prezygotic incompatibility.

Consequently, given that postzygotic incompatibility is sympatrically unstable, there remain two courses of speciation both consisting in the evolution of complete prezygotic incompatibility, one during the phase of reproductive isolation (allopatric) and the other after secondary contact (sympatric). The mechanisms that have to be considered for the first course are largely analogous to those previously discussed for allopatric evolution of postzygotic incompatibility. The second course has to deal with the role that postzygotic incompatibility plays for the establishment of prezygotic incompatibility within a single population.

Models for the establishment of prezygotic incompatibility — Concerning the second course it appears that little is known about mating systems leading to prezygotic incompatibility, the establishment of which reinforces the required reproductive isolation. An exception can be found in a model involving two gene loci with two alleles each, which was proposed by Maynard Smith [1966] and analyzed in more detail by Udovic [1980]. One locus is responsible for viability selection with heterozygote disadvantage, and the other locus determines positive assortative mating. The model allows for the existence of a (locally) stable two-locus polymorphism corresponding to the sympatric existence of two populations whose hybrids show postzygotic incompatibility, and where positive assortative mating takes the place of prezygotic incompatibility. Yet, in this model stable underdominance is assumed to be guaranteed by negatively frequency-dependent viability selection, and the problem of establishment of assortative mating is not considered. Hence, in the strict sense, the model is not one of speciation, since it cannot explain the reinforcement of reproductive isolation, because it neither allows for instability of the polymorphism caused by underdominance nor does it consider the problem of establishment of positive assortative mating.

A more basic criticism of this type of model refers to the fact that the genotypes at one locus are both **determinant** and **object of mating***, whereas viability or fertility selection takes place independently at a second locus. Selection and mating thus interact only via linkage between the two loci. On the other hand, for the present pathway of speciation, the two reproductively isolated populations must be assumed to have diverged genetically, and these genetic differences must determine the characteristics distinguishing the species arising from the two populations. Therefore, both postzygotic selection and positive assortative mating should instead somehow be related to these characteristics. Mating relations (associated with one locus) should thus be defined for the same traits that affect viability or fertility (associated with a second locus or with particular alleles at the first locus), and these traits should simultaneously identify the affiliation to one of the two populations.

The type of model, where determinant and object of mating are separated in that one locus determines the mating relations with respect to a trait coded for by another locus, was already mentioned by Maynard Smith [1966]. Since, as this author pointed out, the effect of the mating determinant consists in modifying the mating relations realized for the object trait it is justified to address it in short as **mating modifier**. Moreover, the situation in which determinant and object of mating are separated may consistently be called **mating modification**. It is probably worth noting that mating modification may involve selection at the object trait (locus) as well as at the modifying trait (locus) itself. The selection at the object locus is likely to be a direct consequence of the mating system resulting in differential mating success [see Gregorius 1989].

Thus, the sympatric establishment of prezygotic incompatibility may in principle be affected by different forces of selection, one producing underdominance for the object trait and the other directly selecting the mating modification to become established. Apparently this genetic model has, however, not attracted much interest in the population genetic theory of mating systems. Only recently was the model of mating modification reexamined by Sanderson [1989], without allowing for selection at the modifier locus and for a system of mating that has probably little biological relevance (as the author himself notes). The additional assumption of constant viability selection with underdominance again led to the failure of speciation by reinforcement. An earlier analysis by Endler [1977, p. 142ff] was partially successful, but again only at the cost of ignoring the possibility of associations between the loci and problems of maintaining the biallelic polymorphism at the selected locus.

The above distinction between determinant and object of mating systems is probably very useful in the classification of the genetic mechanisms giving rise to these systems, where problems of establishment of certain mating relations carry decisive evolutionary biological weight. To demonstrate this point, first consider a gametophytic system of homogenic incompatibility in which determinant and object of the implied mating relations are to be verified. In this system the products of a gene inhibit pollen tube growth if it appears in the pollen and the style tissue, for example. Clearly, the products of this gene

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* The states of a trait are termed objects of mating, if mating preferences exist among them; a possibly different trait, the states of which determine these mating relations, is called a determinant of mating

both directly determine the mating relations and are themselves the object of mating; since determinant and object of mating are identical, the absence of mating modification can thus be postulated, and a genetic model based on a single gene locus could already produce such a type of mating system.

This is different, for example, with positive assortative mating for plumage coloration in populations of the lesser snow goose [see e.g. Cooke et al. 1976], where the trait "plumage coloration" is the object of mating. However, as the authors argued, the forces determining that individuals with like colour preferentially mate with each other may be of completely different origin. Hence, determinant and object of mating are separated, and the evolutionary significance of this fact becomes apparent when studying the establishment of positive assortative mating. In the most simple case, the object and determinant of mating are controlled by one gene locus each, the object locus is polymorphic, and the locus modifying the mating relations is fixed for an allele that allows for random mating with respect to the object trait. Mutants at the latter locus, the modifier locus, may then have different effects ranging from positive assortative mating for some of the expressions of the object trait to such mating for all of the expressions. If this mutant spreads in the population and ultimately becomes fixed, then all individuals concerned show the determination for positive assortative mating, in which case it would be difficult to decide on whether or not the object of mating also is its determinant, i.e. on whether or not mating modification is involved.

Distinction between pathways of speciation — Returning to the mechanisms inherent in speciation by reinforcement, the above considerations suggest that this course of speciation be primarily characterized by the necessity of secondary contact of two previously reproductively isolated populations in order to allow for the evolution of prezygotic incompatibility between these two populations. The prezygotic incompatibility is required to operate on polymorphic (at least dimorphic) traits distinguishing the two populations and is therefore very likely to be determined by gene loci not participating in the expression of these traits. Whether the evolution of postzygotic incompatibility (rather than hybrid advantage, for example) during the phase of reproductive isolation fosters the establishment of prezygotic incompatibility after secondary contact is an open question.

Even under this restrictive definition it might be difficult to experimentally tell speciation by reinforcement from sympatric speciation if the latter pathway proceeds by the evolution of prezygotic incompatibility between parts of the ancestor population. In fact, sympatric speciation does not exclude the possibility that prior to the initiation of speciation the population was split into reproductively isolated parts. Hence, in such a case the two pathways can be distinguished only if the preceeding separation can be proven to have provided the prerequisites for the establishment of prezygotic incompatibility. However, as was mentioned earlier, knowledge about mechanisms that could create such processes still seems to be quite vague.

In principle, the same uncertainty exists with respect to reinforcement and allopatric speciation. That allopatric speciation has actually occurred shows up of course only after cessation of the reproductive isolation and thus in sympatry. The problem is then just reversed, in that one has to exclude the possibility that the reproductive incompatibility arose as a consequence of the cessation of reproductive isolation. Since hybridization is the only experimental tool available for studies aiming at a distinction between pathways of speciation, one can only expect insight in cases where the reproductive isolation or prezygotic incompatibility is not obligatory. Speciation by reinforcement can then be ruled out if no substantial postzygotic incompatibility shows up in the hybrids. Yet, there seems to be little if any evidence for the existence of species that are properly separated by prezygotic incompatibility but show no signs of depression among their (rarely occurring) hybrids. It is probably this co-occurrence of pre- and postzygotic incompatibility that, despite all criticism, explains the continuing popularity of the concept of speciation by reinforcement. On the other hand, the above considerations also explain that on reasons of observability it ought to be difficult to find evidence for speciation by reinforcement in wild populations (see also Sauer, 1990, for particular reference to the detectability of allopatric speciation). However, this of course does not justify the assumption that this pathway of speciation is unlikely in nature.

Crucial features of genetic models of speciation

The considerations in the previous section demonstrate that if in any of the pathways of speciation a critical step occurs, it is either concerned with the persistence of hybrid disadvantage (postzygotic incompatibility) in the absence of reproductive isolation between the hybridizing populations or with the establishment of mating systems guarding against the production of hybrids (prezygotic incompatibility). Interestingly, both these aspects relate to the sympatric mode of speciation, which is generally considered to be most unlikely.

With reference to speciation by reinforcement, the key to understanding seems to be found in the conditions, if there are any, under which two postzygotically incompatible populations can coexist so as to allow for successive establishment of prezygotic incompatibility between these populations without endangering their persistence. By the present definition of population and biological species, speciation among the two populations necessarily entails genetic (and thus genealogical) disjunction in the sense that for at least one gene locus they have no alleles in common. Hence, in the most simple case the two alleles A_1 and A_2 at a biallelic gene locus distinguish between the two populations such that the homozygote A_1A_1 is characteristic of the one and the homozygote A_2A_2 of the other population, whereas the heterozygote A_1A_2 is the product of hybridization between the two. Clearly, before speciation is completed, both populations are to be considered as subpopulations of a single population so that all three genotypes may occur in both subpopulations.

Denoting by w_{11}, w_{12}, and w_{22} the **fitnesses** (= number of successful gametes, i.e. gametes which entered into zygotes) of the three corresponding genotypes, hybrid disadvantage is defined by the "underdominance" relationship $w_{11} > w_{12} < w_{22}$, where the fitnesses are taken over the whole population (i.e. both subpopulations). For complete postzygotic incompatibility between A_1A_1 and A_2A_2 genotypes, $w_{12} = 0$. Effects of the mating system (including migration or gene flow between subpopulations) can be accounted for by specification of the fractions of the fitness of a genotype which it owes to matings with each of the other genotypes, including its own. These quantities were termed **fractional fitnesses** by the present author [Gregorius 1984a]. If x and y denote any two different genotypes of the above three, then the fractional fitness $w_{x \triangleleft y}$ of genotype y with respect to genotype x is defined to be the average number of successful gametes of an individual with genotype y that result from fertilization by individuals with genotype x. Similarly, $w_{y \triangleleft y}$ is one-half the average number of successful gametes of an individual with genotype y resulting from fertilization by the same genotype. Note that $w_y = 2 \cdot w_{y \triangleleft y} + \sum_{x: x \neq y} w_{x \triangleleft y}$.

To enable separation of effects of fitnesses from those of mating systems on the dynamics of genotypic frequencies, it is useful to apply the fitness fractions $f_{y \triangleleft y} := 2 \cdot w_{y \triangleleft y} / w_y$ and $f_{x \triangleleft y} = w_{x \triangleleft y} / w_y$ for $x \neq y$, so that $\sum_x f_{x \triangleleft y} = 1$. These fractions can also be referred to as **conditional mating frequencies**. Under the biologically reasonable assumption that the fractional fitnesses are continuous functions of the genotypic frequencies (extending to the boundaries where some of the frequencies become 0), the present author [Gregorius 1984b] proved that for underdominance the allele A_1 cannot become established and is thus not protected if $w_{11} \leq w_{22}$ for small frequencies of A_1. For $w_{11} > w_{22}$ the allele is protected if the following inequality holds true for very small frequencies of A_1:

$$f_{11 \triangleleft 11} > \frac{w_{22}}{w_{11}} - \tfrac{1}{2} f_{12 \triangleleft 12} \cdot \frac{w_{12}}{w_{11}} \cdot \frac{w_{11} - w_{22}}{w_{22} - w_{12}} \qquad (*)$$

A_1 is not protected if this inequality holds in the reverse direction. Analogous results for the allele A_2 are obtained by exchanging the indices 1 and 2 in the above conditions.

To understand the implications of this fairly general result, consider that the conditional mating frequencies describe effects of the mating (or migration, or gene flow) system when compared with the frequencies P_x, say, with which the genotypes x participate in the matings. Thus, $P_x < f_{x \triangleleft y}$ indicates that genotype y mates more frequently with genotype x than under random mating. In particular, if $P_x < f_{x \triangleleft x}$, we are used to speak of positive assortative mating of the genotype x [see Gregorius 1989]. Hence, if $f_{x \triangleleft x}$ remains properly positive as the frequency of A_1 and thus P_x tends to 0, genotype x must practice positive assortative mating when rare. Consequently, according to inequality $(*)$,

> *for underdominance the chances of an allele to become established and be protected increase with increasing amounts of positive assortative mating provided the rare homozygote exceeds the frequent in fitness.*

In order to guarantee that both alleles persist in the population, i.e. that the biallelic polymorphism is protected, it is therefore necessary that the two homozygotes show negative frequency dependence in fitness (the rare homozygote exceeds the frequent in fitness). However, it needs a sufficient amount of positive assortative mating in addition (at least for the rare genotypes) to actually reach the situation of protectedness of the polymorphism (this is not the same as Udovic's [1980] result, where it is not the positive assortative mating but rather the negative frequency dependence that *a priori* stabilizes the polymorphism at the viability selected locus).

In fact, the conditional mating frequencies f appearing in inequality $(*)$ comprise the effects of migration or gene flow between (sub)populations on the mating relations in the total population. To see this, consider that in an effectively subdivided population, individuals by definition assort positively with respect to the subpopulation they belong to. With sufficient genetic differentiation among the subpopulations, this entails *genetically* positive assortative mating, since preferential mating within a subpopulation implies an excess of matings with the locally prevailing (adapted) genotype as compared to the frequency of this genotype in the total population. It is this type of positive assortative mating which, in essence, explains the protectedness of a genetic polymorphism in the absence of heterozygote fitness advantage and which comprises as a special case the common models of selection-migration-balance [see Deakin 1968, or Karlin and McGregor 1972].

The more commonly known condition for protectedness of a biallelic polymorphism is overdominance, i.e. $w_{11} < w_{12} > w_{22}$. It is characteristic that in this case negative frequency dependence in fitness of the two homozygotes is sufficient for a protected polymorphism irrespective of the mating system. However, in the critical situation of positive frequency dependence (the frequent homozygote exceeds the rare in fitness) or constant ranking of the fitnesses, positive assortative mating has the effect of endangering the persistence of the biallelic polymorphism [see Gregorius 1984b]. This, of course, relates to pathways of speciation other than speciation by reinforcement.

Conclusions

Allopatric speciation usually occurs as a consequence of adaptive divergence between populations, and the associated difference in environment (habitat) makes it unlikely that such populations return to a genuinely sympatric existence. Sympatry would give way to competition, which, in turn, entails the danger of competitive exclusion of one of the populations and thus the abolishment of the result of the speciation process. The avoidance of competition during the process of speciation and after its completion probably is the most important characteristic of purely allopatric speciation (see also the discussion of the significance of competition relative to speciation in Levin 1970). In fact, in the other pathways of speciation involving phases of sympatry, negatively frequency dependent selection among the genetic variants defining the prospective species populations appears to be a necessary prerequisite for the persistence of these variants and thus of the new species to be formed. This type of selection helps stabilizing competition relations in sympatry.

Sympatric speciation — Concerning purely sympatric speciation, the model in the last section suggests that (partial) postzygotic incompatibility cannot evolve in the absence of prezygotic incompatibility. Hence, either the evolution of (partial) prezygotic incompatibility precedes that of (partial) postzygotic or both evolve simultaneously (pleiotropically). In the first case the two steps differ in that prezygotic incompatibility also can become established without modification of pre-existing mating relations. An example would be provided by a mutant causing its carriers to only mate among themselves and by this conferring a selective advantage that vanishes with increasing frequency (as is the case with saturation of mating predispositions, see Gregorius 1989, p. 100ff). The same can of course be achieved by selectively advantageous modification of pre-existing mating relations.

The subsequent establishment of postzygotic incompatibility must then operate on the existing polymorphism for the mating trait by modification of the selective values of the expressions of this trait. Further steps towards increasing both types of incompatibility until complete reproductive incompatibility is reached can only be successful if in each step thresholds of the type given in inequality $(*)$ are not violated. This inequality also suggests that sympatric speciation proceeds more safely along series of increases in pre- rather than postzygotic incompatibility. It might, however, be difficult to specify conditions under which a gene can gain a selective advantage and become established by causing postzygotic incompatibility among heterozygotes at other gene loci. Here, the probably most simple type of population genetic model is based on positive assortative mating at one gene locus with a second locus causing heterozygote disadvantage in viability at the first locus.

Pleiotropic evolution of pre- and postzygotic incompatibility is indeed possible, as inequality $(*)$ demonstrates for the simple case of a biallelic gene locus. Here again, the term “pleiotropy” may refer to control of different traits by the same genes or to multiple effects of a single trait. For example, a single change in flower structure may increase both the amount of self-fertilization and the overall fertilization success. In this case one trait pleiotropically affects the mating system and the fitness. Other non-pleiotropic forms of simultaneous evolution of post- and prezygotic incompatibility are probably quite unrealistic, since they must assume some degree of independence between the traits determining pre- and postzygotic incompatibility. This independence would, in turn, require the simultaneous occurrence of functionally completely different mutants whose further joint evolution would more or less be subject to the vagaries of initial conditions.

Speciation by reinforcement and parapatry— The previous discussion and, in particular, the model producing inequality $(*)$ clearly show that the critical phase in this pathway of speciation is characterized by the manifestation of postzygotic incompatibility after secondary contact and the associated risk of extinction of one of the two populations. Inequality $(*)$ states that largely unrestricted hybridization endangers coexistence of the two populations irrespective of whether mating is the only contact realized between the populations. Hence, pre-zygotic incompatibility should evolve at high speed immediately after secondary contact to stop the tendency towards extinction. This is probably a very unrealistic condition and therefore necessitates the search for other conditions that have effects similar to those of prezygotic incompatibility. By the latter it is understood that, at the least, the speed of extinction is slowed down so as to allow for the timely establishment of more efficient forms of prezygotic incompatibility.

In the incipient phases of secondary contact, restricted gene flow among the populations is likely to be the most efficient mechanism for lowering the degree of hybridization. That this parapatric situation may indeed lead to stable coexistence of the two populations was first demonstrated by Levene [1953] and Deakin [1968] and then confirmed in more detail by Karlin and McGregor [1972]. These authors showed that a stable polymorphism can be obtained at a biallelic locus, if at this locus selection acts in opposite directions in two ecological niches and if migration between the two niches is sufficiently low (see also the explanations following inequality ($*$)). It is therefore reasonable to suggest that the chances for speciation by reinforcement increase considerably if, after allopatric evolution of postzygotic incompatibility, secondary contact is initiated by an extended parapatric phase of quite low gene flow between the two populations.

However, this ought to be substantiated, for example, by the analysis of two-locus models in which a genetic polymorphism is maintained at one locus by low migration between two niches with underdominant selection operating in opposite directions in both, and where a mutant at the second (mating modifier) locus introduces positive assortative mating or cross-incompatibility at the first. The mutant modifying the mating relationships may, however, also occur at the selected locus, where it could be equivalent to one of the resident alleles but could pleiotropically affect its mating characteristics. Single locus models may thus also be useful tools for analysis [see e.g. Orr 1991].

In summary, from a population genetic point of view it appears that

- ▷ speciation through genuinely sympatric evolution of postzygotic incompatibility (in the absence of prezygotic incompatibility) is probably impossible;
- ▷ allopatric evolution of postzygotic incompatibility alone is an unstable pathway of speciation in the sense that secondary reproductive contact can easily lead to ultimate extinction of one of the two species populations;
- ▷ allopatric, sympatric, or parapatric evolution of prezygotic incompatibility, if they take place, seem to be the only stable pathways of speciation;
- ▷ it is still unclear whether sympatric evolution of prezygotic incompatibility can more readily be initiated in the presence or in the absence of (partial) postzygotic incompatibility;
- ▷ in the presence of postzygotic incompatibility the chances for prezygotic incompatibility to evolve after secondary reproductive contact (reinforcement) increase, if this contact is initiated by an extended phase of small amounts of gene flow between the populations (parapatry);
- ▷ the sympatric evolution of partial prezygotic incompatibility may facilitate the subsequent establishment of postzygotic incompatibility;
- ▷ more insight into processes of speciation can be expected from population genetic studies which analyze conditions for the establishment of pre- and postzygotic incompatibility and which are built on the concept that the traits distinguishing species are also the ones which are simultaneously objects of assortative mating and of disruptive selection during speciation.

In a recent review on pathways of speciation in *Drosophila* species, Coyne and Orr [1989] addressed several of the above items by measuring amounts of pre- and postzygotic incompatibility. Despite their own criticism as to the appropriateness of the measures applied, the observations largely confirm the predictions of the present theories. Among the main statements of Coyne and Orr are that (*i*) for sympatric species the fraction of cases where prezygotic incompatibility is considerably stronger than postzygotic incompatibility exceeds by far that fraction for allopatric species, (*ii*) prezygotic incompatibility occurred between all pairs of species studied, but in several cases postzygotic incompatibility was not detectable, (*iii*) among those pairs of species showing no postzygotic incompatibility, sympatric pairs are much more frequent and exhibit larger degrees of prezygotic incompatibility than allopatric pairs, and that (*iv*) there are allopatric species showing no signs of postzygotic incompatibility as follows from (*iii*).

Statement (*i*) is in agreement with the finding that in the presence of postzygotic incompatibility the chances for sympatric speciation to proceed successfully increase with increasing amounts of positive assortative mating (prezygotic incompatibility). The suggestion that prezygotic incompatibility is the stabilizing factor in all modes of speciation is supported by statements (*ii*) and (*iv*). Moreover, (*ii*) corroborates the result that postzygotic incompatibility alone is unlikely to maintain stable pathways of speciation. Since about equal numbers of sympatric and allopatric pairs of species were included in the study, statement (*iii*) implies that, if speciation takes place in the absence of postzygotic incompatibility at all, it is more likely to do so in sympatry than in allopatry. This is in accordance with the fact that selection for prezygotic incompatibility can act in sympatry but not in allopatry, which attributes the evolution of prezygotic incompatibility in allopatry to the more or less accidental emergence of conducive adaptive environmental differences.

All of these considerations refer to the situation where speciation has taken place and where thus the relative importance of the various pathways of speciation is to be evaluated. Whether or not speciation is a frequently occurring evolutionary process and which pathway predominates must be decided on a different basis. Among others this requires characterization of environmental conditions (including inter- and intraspecific interactions) and genetic systems that either inhibit or foster the evolution of pre- and/or postzygotic incompatibility. Concerning genetic systems, the presently adopted approach suggests that the conditions be specified which allow for or inhibit the establishment of genes or gene complexes that participate in the realization of the various forms of incompatibility. The resulting models, based on theoretical or experimental reasoning, may then help to simplify the formulation of testable hypotheses.

An illustrative example for a genetic model based on experimental reasoning is provided by the work of Tauber and Tauber [1977], who studied speciation through habitat diversification and seasonal reproductive isolation in the two green lacewings *Chrysopa downesi* and *Chrysopa carnea*. From their investigations they concluded that coloration of *C. downesi* is due primarily to a single, semidominant autosomal allele, and that the difference in coloration between the two species corresponds to the adaptive requirements (camouflage) of their habitats. Moreover, the seasonal reproductive asynchrony of the two species (non-overlapping univoltism and multivoltism) can be traced back to a single allele difference at each of two loci, and this difference in reproductive behaviour again constitutes an adaptation to the two habitats. Clearly, the conditions that allowed establishment of the coloration mutant and the two mutants required for seasonal reproductive isolation escape experimental verification and are therefore only a subject for speculative reasoning.

The authors propose a sympatric pathway of speciation in which disruptive selection for coloration between the two habitats produces heterozygote disadvantage and where seasonal reproductive isolation subsequently reduces the production of disadvantageous heterozygotes. This hypothesis faces the problem of instability associated with underdominance and random mating. On the other hand, since the traits implying reproductive isolation are adaptations to different habitats in the first place, they could just as well have evolved prior to the coloration polymorphism, and, because of the then reduced gene flow between the habitats, could thus have improved the chances for establishment of the coloration polymorphism. However, the fact that the coloration alleles show semidominance induced the authors to postulate intermediate fitnesses of the heterozygotes in both habitats, so that underdominance need not be realized across habitats under all frequency conditions. This would, in turn, counter the criticism of the hypothesis that the coloration polymorphism became established first. In any case, the genes involved in the reproductive isolation may be considered to act pleiotropically on the fitness of their carriers (as was pointed out earlier) in the two habitats. It is probably the latter that is most characteristic of the pathway of speciation of the two lacewing species studied.

It should be emphasized that Tauber and Tauber [1977] found no signs of pre- or postzygotic incompatibility between *C. carnea* and *C. downesi* in laboratory tests. Thus, despite the sympatric existence over wide ranges of the two species, gene flow between them is obviated by the reproductive isolation resulting from the asynchrony in their reproductive cycles. In effect, this situation is tantamount to that of reproductively isolated allopatric populations which have not yet evolved any reproductive incompatibility, and which would therefore not have been considered as belonging to different biological species. The observations of the authors would, under this point of view, have been classified as adaptation to different niches or habitats rather than speciation. In fact, if one accepts laboratory conditions as environments under which a test of conspecificity of two populations is meaningful, the concept of biological species, as restated in the Introduction, would yield conspecificity of *C. carnea* and *C. downesi.* On the other hand, if the "conditions" for tests of biological conspecificity are restricted to natural habitats to which the populations are specifically adapted, *C. carnea* and *C. downesi* are to be classified as different biological species, since under these conditions they apparently were never observed to fulfill the requirements of a single Mendelian population.

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