Syst. Biol. 41(4):421-435, 1992

POPULATIONS, GENETIC VARIATION, AND THE DELIMITATION OF PHYLOGENETIC SPECIES

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Abstract.—Systematists should distinguish between cladistic analysis, i.e., a numerical procedure, and phylogenetic analysis, i.e., the reconstruction of hierarchic descent relationships. Modern cladistic analysis uses parsimony to construct hierarchic arrangements (trees) of terminal units (terminals) that have been scored for a series of attributes. The interpretation of cladistic relationships as representative of phylogenetic relationships requires two conditions, both of which were identified by Hennig (1966, Phylogenetic systematics, Univ. Illinois Press, Urbana). First, descent relationships among the terminals must be hierarchic; that is, all terminals must have been generated by the subdivision or replication of previously existing ancestors. This is a necessary condition for phylogenetic analysis, rather than an empirical discovery of it, because the results of cladistic analysis are always hierarchically structured (however poorly resolved). Because resolution of a cladistic hierarchy does not demonstrate that a hierarchic descent system underlies the character distribution pattern it reflects, additional information is necessary, in any given case, to determine that phylogenetic analysis is appropriate. Second, to have congruence between an observable attribute hierarchy and the descent hierarchy that is to be inferred, the attribute must have been transmitted from an ancestor to all of its descendants, either in its original state or in a modified state. Both conditions are met by asexual organisms and by nonrecombining genetic elements (e.g., the chloroplast and mitochondrial genomes) but not by individual sexually reproducing organisms that bear such genetic elements. Populations of sexually reproducing organisms can meet the first condition (i.e., hierarchic descent) when new populations are founded by the division of previously existing populations and individuals do not disperse among existing populations. When the first condition is met, the second also is met for genetically fixed attributes of the populations, because populations descended from an ancestral population that was fixed for an attribute also will be fixed for that attribute (in original or modified state). In contrast, attributes that are not fixed in a population may not occur in all or any descendant populations, even if descent relationships among populations are hierarchic. The occurrence of a unique fixed character combination in an extended genealogical population (phylogenetic species sensu Nixon and Wheeler, 1990, Cladistics 6:211-223) is evidence that this population has diverged from other such populations and thus that descent relationships among such populations are hierarchic. The fixed characters of phylogenetic species therefore constitute evidence that a hierarchic descent system exists and provide the means for analyzing phylogenetic relationships among these species. Phylogenetic species can be delimited by a procedure (population aggregation analysis) that involves a search for fixed differences among local populations, followed by successive rounds of aggregation of populations and previously aggregated population groups that are not distinct from each other. [Cladistics; phylogenetics; hierarchy; phylogenetic species; population variation.]

Cladistic analysis has become widely accepted as the most rigorous approach to the study of phylogenetic relationships. Its success is attributable in large part to the careful attention given by its proponents to the assumptions and logical foundations of the method (e.g., Hennig, 1966; Nelson and Platnick, 1981; Farris, 1983). In this paper, we discuss some of the assumptions that pertain to phylogenetic analysis, specifically those relating to the minimal units appropriately employed as terminal units (or simply "terminals"). The goal of most practitioners of cladistic analysis is to reconstruct descent relationships among biological terminals. We argue that phylogenetic analysis, as an application of cladistic analysis, is meaningful only with terminals that meet specific conditions; that Hennig identified these conditions when he formulated his phylogenetic method; and that many analyses conducted in recent years are flawed by the indiscriminate application of phylogenetic reasoning to analyses of terminals that do not meet these conditions. There are means available for

the identification of terminals that meet the necessary conditions and thus are appropriate for phylogenetic analysis.

The Problem: Cladistic vs. Phylogenetic Analysis

We begin by differentiating cladistic analysis from phylogenetic analysis. Modern cladistic analysis is a parsimony-based search for hierarchic arrangements (trees) of terminal units (terminals) that have been scored for a series of attributes. As a strictly numerical procedure, there is no requirement in cladistic analysis for a background model that relates these trees to biological processes, such as reproduction or descent.

Phylogenetic analysis, in contrast, is the reconstruction of descent relationships among terminals. Although the term "phylogenetics" can refer to both hierarchic and nonhierarchic (e.g., polyploid) descent relationships, we confine our usage here to its more common application, to hierarchic descent systems. Phylogenetic relationships typically are reconstructed using parsimony analysis of the distribution of attributes (characteristics) among the biological terminals. The use of cladistics to infer phylogenetic relationships does not make the two equivalent, however, because reconstruction of a descent history involves assumptions that are unnecessary for a numerical procedure alone.

Hennig (1966) initially described phylogenetic analysis as a means of reconstructing hierarchic descent relationships *among species*. His explicit reference to species was intended as a restrictive condition, as evidenced by his detailed discussion of reticulate (i.e., tokogenetic, parent-offspring) descent relationships, which exist among individuals of a sexually reproducing population, as opposed to hierarchic (i.e., phylogenetic) descent relationships, which exist among certain groups of organisms (i.e., species). A similar point has been made more recently by Brady (1983), who considered hierarchy an operational assumption of phylogenetic analysis.

If phylogenetic analysis is properly applied only to systems of hierarchic descent, can the results of a cladistic analysis be used to determine that this condition is met in a given case? Consider a case in which panmictic populations I and II are distinguished by a fixed difference (allele P vs. allele Q) at a nuclear locus (Fig. 1). At a separate locus, a nonrecombining maternally inherited gene (borne on the mitochondrial or chloroplast genome), there are four alleles, O, A, B, and C; one genotype exists in population I, and three exist in population II (Figs. 1a, 1b). A fully resolved gene tree reflecting relationships among these four alleles is constructed on the basis of restriction site or sequence variation, and when this gene tree is rooted with allele O, alleles A, B, and C are identified as a nested set of states (Fig. 1c). A cladistic analysis of variation in population II is conducted; groups of individuals of identical genotype constitute the ingroup terminals, and the sole genotype in population I is used as the outgroup. A fully resolved cladogram again is generated (Fig. 1d). If this cladogram is interpreted as reflective of phylogenetic relationships, terminals II_B and II_C are regarded as collectively constituting a monophyletic group whose members share a more recent common ancestor with each other than either does with individuals of group II_A. However, this interpretation is incorrect, because population II is panmictic, and membership in each of the three genotypic classes (II_A, II_B, and II_C) is random with respect to the genotype of each individual's male parent; two individuals with different genotypes may share a more recent common ancestor with each other (e.g., the same father) than either does with other individuals of its own genotype. The flaw in this analysis is not in the cladogram but in the interpretation of a cladistic pattern as reflective of phylogenetic relationship. Although the cladistic structure may represent descent relationships among alleles, it in fact depicts phenetic "relationships" among the genotypic classes of individuals within population II. Hence, the ability to generate a resolved cladogram of attributes borne by organisms does not demonstrate

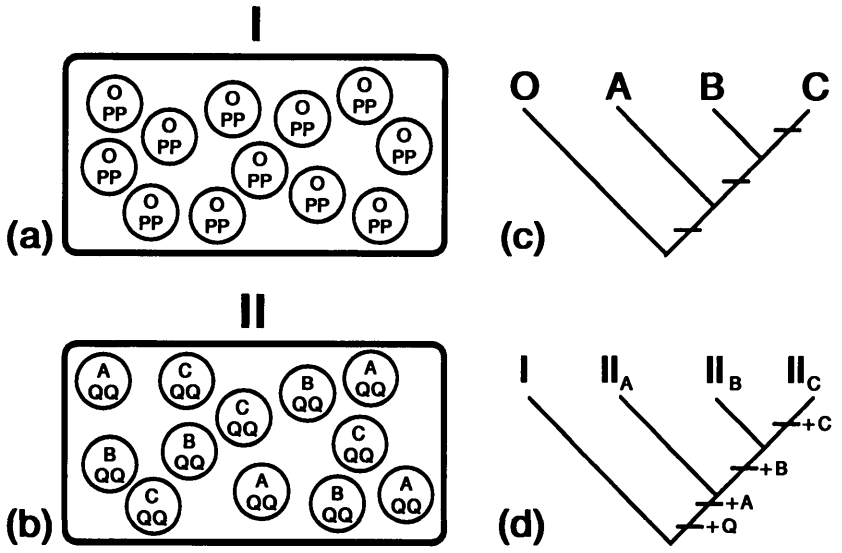

FIGURE 1. (a), (b) Two panmictic populations with individual organisms labeled by genotype. P and Q are alleles of a nuclear gene; O, A, B, and C are alleles of a maternally inherited gene. Population I is fixed for allele P at locus P/Q and for allele O at the maternally inherited locus. Population II is fixed for allele Q at locus P/Q and is polymorphic for alleles A, B, and C at the maternally inherited locus; it therefore comprises three genotypic classes, II_A, II_B, and II_C. (c) Gene tree depicting cladistic relationships among the four alleles of the maternally inherited gene. Three characters supporting the structure of the tree (e.g., restriction site mutations) are plotted on the cladogram but not individually labeled. (d) Single most-parsimonious cladogram depicting variation among the four genotypic classes of individuals in populations I and II.

that the terminals of the analysis are elements of a hierarchic descent system.

If we depart from the extreme case of a panmictic population, we discover that phylogenetic reasoning frequently is invoked in situations that are not qualitatively different. Some investigators have explicitly promoted the use of individuals as terminals (Vrana and Wheeler, 1992), whereas others have suggested that populations are appropriate terminals, if relationships among them are "predominantly diverging" (de Queiroz and Donoghue, 1988:326). Still others have drawn phylogenetic conclusions (i.e., concerning relationships among groups of individuals) from cladistic analyses of allelic variants that coexist within species (e.g., Avise et al., 1987; Vigilant et al., 1989, 1991).

There are, of course, intermediate cases in which relationships among individual organisms are predominantly hierarchic, with rare occurrences of sexual recombination (e.g., see Mishler, 1990). Despite the existence of such cases, Hennig's contrast between phylogenetic and tokogenetic descent systems is fundamental, and systematists should be attentive to this problem. Consider again the example depicted in Figure 1. If, rather than being drawn from a single panmictic population, the individuals of groups II_A, II_B, and II_C represented three different populations, each nearly fixed for a different allele, but each carrying all three alleles, would phylogenetic interpretations of the resulting cladogram then be valid? One can choose either to assume without evidence that phylogenetic analysis is appropriate in a given case or to devise criteria to determine when it is. Searching for evidence of hierarchy is the more prudent course, and an appropriate criterion is available in the form of fixation of alternative characters in different population systems.

HENNIG'S MODEL

In the following discussion we use the terminology of Nixon and Wheeler (1990), who distinguished between characters and

traits. A character is an attribute that is invariable (i.e., fixed) within a terminal lineage or a monophyletic group. By "fixed," we do not mean that the attribute is necessarily observed as monomorphic but that it occurs in all individuals of the lineage, in either its original state or in a transformed state. In contrast to characters, *traits* are attributes that occur in some but not all representatives of a terminal lineage or monophyletic group. Consider a species that at one time is fixed at some locus for allele A (hence a character). At a later time, a mutant form of this allele arises (i.e., allele B, a transformed state of A). A and B now are alternative alleles at the locus. Both may appear to be traits to an investigator, but allele A (in original or transformed state) actually remains a character, whereas allele B is a trait. Should allele B later become fixed, it too will be a character, and allele A, although seemingly absent, will remain a character. Under these definitions, the genes encoding hemoglobins are characters of the human species, whereas most (perhaps all) of the alleles at the ABO blood group locus are traits. We emphasize that these concepts of character and trait are theoretical and that it may not always be possible to distinguish the two by direct observation, just as presence of limbs is regarded as a synapomorphy of all tetrapods despite the apparent absence of limbs in some species of this group. We further emphasize that character fixation in this context is a historical concept; a plesiomorphic character remains fixed even after transformed states have appeared and perhaps replaced it. Also, a character may be fixed in a population although it is not directly observable in all individuals or at all developmental stages; thus, age-specific and gender-specific attributes can be characters. In adopting this distinction between character and trait, we further specify the use of *attribute* as a collective term for the two or to refer in empirical studies to characteristics of undetermined status.

Hennig (1966) described phylogenetic analysis as a method for the discovery of descent relationships among species. He presented this restriction in the context of a contrast between tokogenetic and phylogenetic relationships; he regarded species as appropriate terminals for phylogenetic analysis because he regarded them as related to each other hierarchically. For the sake of the present discussion, it is less important to gain a precise understanding of Hennig's species concept than it is to clarify the relationship between hierarchic descent and phylogenetic inference. Hennig (1966, cf. pp. 88–93) justified phylogenetic inference as a "scheme of argumentation" that involved hierarchic descent and character evolution, and he described conditions that constitute the crucial link between character distribution patterns and descent relationships. We refer to these conditions as Hennig's model (Nixon and Wheeler, 1992).

The first condition of this model is that descent relationships among the terminal units of the analysis are hierarchic. That is, terminals arise only by the division or replication of previously existing ancestors; reticulate descent does not occur. It follows that for any specified set of terminals there is just one most recent common ancestor (MRCA) of all members of the set (Hennig, 1966; Farris, 1974; Nixon, 1993). The existence of a process of hierarchic descent, which generates lineages with unique histories, is not dependent upon the ability of investigators to detect it.

The second condition of Hennig's model, which we regard as implicit in his discussion, is that all descendants of a common ancestor within a hierarchic descent system retain all of the ancestor's characters, either in original or transformed state. This condition constitutes the crucial link between character distributions, which are observable, and the underlying descent history that is to be inferred. Although many (most?) lineage divergences may not be marked by character changes and thus are not discoverable by cladistic analysis, the second condition allows some portion of the descent history to be reconstructed because it allows the events that are marked by character change to be recovered. In contrast, if descendants do not retain all

attributes of ancestors (e.g., in sexual systems), there is no necessary relationship (after a very few generations) between descent relationships and trait distributions.

Given this theoretical model, is it possible to specify which (if any) biological systems fulfill its conditions? We first consider asexual organisms, in which reproduction is uniparental (clonal). For such organisms, the first condition of Hennig's model is met: for any set of individuals there is one MRCA, because descent is hierarchic. The second condition also is met, because every descendant of an individual bears each of the ancestor's characters, in original or transformed state. A portion of the descent history of individual organisms (that part marked by mutations) is reflected in a hierarchic character distribution pattern and is recoverable by cladistic analysis. Indeed, the reconstruction of gene trees for nonrecombining genetic elements (e.g., chloroplast DNA molecules) is justified specifically in this manner, although the interpretation of such gene trees as representative of species phylogenies requires additional assumptions (Takahata and Nei, 1985; Neigel and Avise, 1986; Doyle, 1992).

In sexual organisms, descent relationships among individuals are not hierarchic; two individuals can have one to many MRCAs. Accordingly, the distributions of traits within sexual populations are not necessarily hierarchic; a trait that is borne by one individual may be borne by all, some, or none of its descendants (Fig. 2) (Mendel, 1866; Hennig, 1966; see also Nixon and Wheeler, 1990, 1992). Because neither condition of Hennig's model is met for sexually reproducing individuals, cladistic patterns of trait variation do not reflect phylogenetic relationships.

Although descent relationships among individuals within sexual populations are not hierarchic, these relationships may be hierarchic among populations or lineages of sexual individuals; when they are, the first condition of Hennig's model has been met. Furthermore, these hierarchic descent relationships may be recoverable by cladistic analysis of character distribution patterns if the second condition of Hennig's model also is met. Thus, phylogenetic analysis may be possible under particular circumstances.

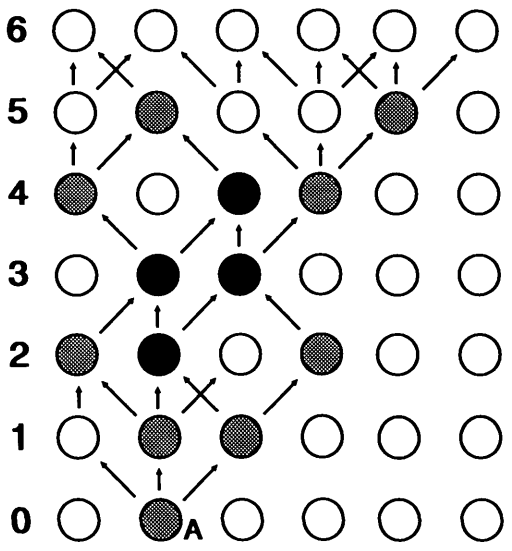

FIGURE 2. Occurrence of a trait (heterozygotes represented by cross-hatching, homozygotes by black shading) through seven generations (0–6) in a sexual population. Parent–offspring relationships are depicted for lines of descent originating from the initial attribute bearer (individual A). Individual A is an ancestor of all individuals that bear the trait. Some but not all descendants of individual A in generations 1–5 bear the trait. In generation 6, the trait has been lost from the population; every individual in the population is now a descendant of individual A but none of these descendants carries the trait.

First, hierarchic descent relationships exist when all reproduction occurs by replication or "divergence" events (e.g., cladogenesis). This condition is satisfied for populations of sexually reproducing individuals (not for the individuals themselves) when new populations are founded exclusively by the fragmentation of previously existing ones. Each of these fragmentation events is the subdivision of an ancestral population into two or more descendant populations. After a fragmentation event occurs, all of the populations that remain are descendants of the original population, regardless of the relative numbers of individuals in these populations and of the physical location that each occupies (Lidén, 1990). When fragmentation

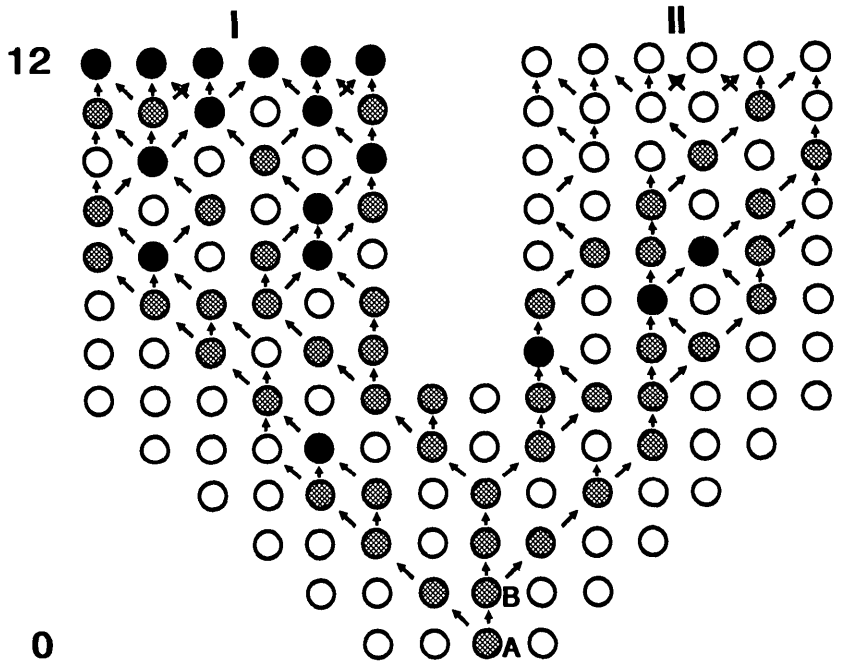

FIGURE 3. Occurrence of an attribute (heterozygotes represented by cross-hatching, homozygotes by black shading) through 13 generations (0–12) as an ancestral population diverges to yield two descendant populations (I and II). Parent-offspring relationships are depicted for lines of descent originating from the initial attribute bearer (individual A). In generation 12, individual A is an ancestor of all individuals in populations I and II; in the same generation, individual B is an ancestor of all individuals in population II and five of the six individuals in population I (it is not an ancestor of the leftmost individual). Thus, in generation 12, five individuals of population I share a more recent common ancestor with all individuals of population II than do all individuals in population I with each other. In generation 12, the attribute becomes a character of population I and is lost from population II; the two populations now are phylogenetic species, neither of which is "monophyletic" or "paraphyletic" (see text).

takes the form of a few individuals dispersing from a large population, the population that remains at the original location appears to retain its physical and genetic integrity and frequently is regarded as the "ancestor" of the distant population founded by the dispersal event. Actually, the population that seems to remain in place is as much a descendant of the common ancestor as is the smaller population at the remote site, for each carries a complete or partial subset of the ancestral gene pool. The remote population may retain alleles that the other does not. Once a new population is founded, the condition of hierarchic descent is violated if gene flow later occurs between it and another. We emphasize this point to stress that it is incumbent upon those who seek to reconstruct intraspecific phylogenetic pattern either to provide evidence that relationships among the terminals of their analyses are hierarchic or to reject the first condition of Hennig's model as a requirement for phylogenetic analysis.

The second condition of Hennig's model also may be met when sexual populations are the units of analysis. Here the distinction between traits and characters is critical. When populations of sexual organisms generate new populations exclusively by division, the second condition is met for the ancestral population's characters but not necessarily for its traits (Fig. 3). Once a population becomes fixed for an attribute (hence a character), all populations descended from this one also are fixed for this character, as long as the descent system remains hierarchic. In contrast, the traits of a sexual population (like any nonfixed

alleles carried in a sexually reproducing individual; see Fig. 2) may occur in all, some, or none of its descendant populations, even if descent relationships among populations are hierarchic (Fig. 3).

The existence of reticulate descent relationships within hierarchically related units (i.e., among sexually reproducing individuals within each population of a nonreticulating population system) does not prevent the first condition of Hennig's model from being satisfied by the terminals themselves. Hence, it is possible for both conditions of Hennig's model to apply to systems of populations of sexual individuals, and when these conditions are met it is appropriate to reconstruct phylogenetic relationships among the populations. Cladistic analysis of such populations can recover those steps of the actual descent history that are marked by character fixation events.

We have indicated that the lowermost point at which hierarchic descent relationships are discoverable by cladistic analysis is the point at which hierarchically related units exist and are marked by characters. Even in a hierarchic descent system, the transmission of traits is not always hierarchic, so the least inclusive units for which phylogenetic pattern is recoverable are those that are marked by fixed differences, i.e., characters. Below this point there may be any number of levels of actually hierarchic descent relationships, but because they are not marked by characters they are not discoverable by cladistic analysis.

The Phylogenetic Species Concept

The phylogenetic species concept (PSC) was discussed by Nixon and Wheeler (1990), who amplified upon earlier contributions by Eldredge and Cracraft (1980), Nelson and Platnick (1981), Cracraft (1983, 1989), and others. Nixon and Wheeler (1990:218) defined a phylogenetic species as "the smallest aggregation of populations (sexual) or lineages (asexual) diagnosable by a unique combination of character states in comparable individuals (semaphoronts)." This definition is congruent with the conditions we have just asserted as necessary for phylogenetic analysis; the PSC was proposed specifically as a framework for the discovery of minimal terminals for phylogenetic analysis.

In contrast to the PSC as defined above, a fundamentally different "phylogenetic species concept" that requires autapomorphies for the diagnosis of species was proposed by Rosen (1979) and has been embraced, in various manifestations, by a number of authors (e.g., Hill and Crane, 1982; Mishler and Donoghue, 1982; Donoghue, 1985; Mishler, 1985; Mishler and Brandon, 1987; de Queiroz and Donoghue, 1988). Although our present goal is not to provide a comparative analysis of these alternative concepts (see Nixon and Wheeler, 1990, and citations therein), some comment is necessary. The term "phylogenetic" as a designator for Nixon and Wheeler's PSC specifies the role of the species to which it refers as the least inclusive units appropriately used as terminals in phylogenetic analysis. Consistent with the view that these are the minimal units among which there is evidence of hierarchic descent relationship, phylogenetic analysis using less inclusive units as terminals cannot be conducted to discover phylogenetic species. Species delimitation, like character-state definition, is a preliminary activity of phylogenetic analysis, and both of these elements, characters and terminals, are the fundamental components of which hypotheses of phylogenetic relationship are constructed. In light of the procedural sequence implicit in the use of the PSC—first the delimitation of species, then the analysis of relationships among them—it is fundamentally different in nature from any "phylogenetic species concept" that involves a prior phylogenetic analysis of less inclusive terminals (e.g., individuals, local populations) followed by species delimitation on the basis of the results.

In its requirement that a phylogenetic species be "diagnosable by a unique combination of character states," the PSC stipulates that every comparable individual of a phylogenetic species carry the entire complement of characters of that species (Fig. 4a). In contrast, if an attribute is fixed

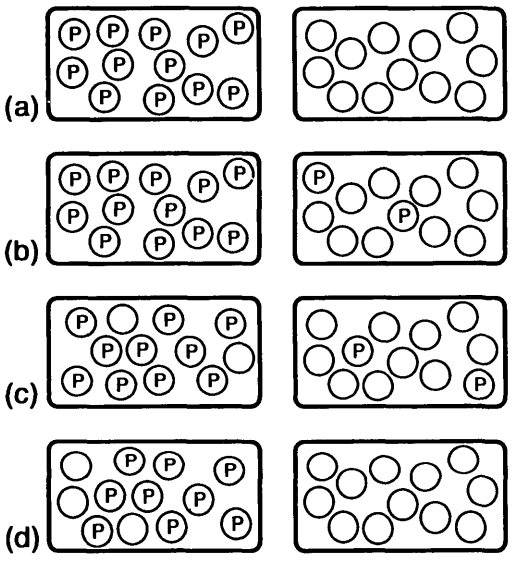

FIGURE 4. Occurrence of a maternally inherited attribute (P) among individuals in four pairs of populations. (a) P is a character of one population but is absent from the other; two phylogenetic species are distinguishable. (b) P is a character of one population and a trait of the other; phylogenetic species are not distinguishable. (c) P is a trait of both populations, which differ in its frequency of occurrence; phylogenetic species are not distinguishable. (d) P is a trait of one population but does not occur in the other; phylogenetic species are not distinguishable.

in one population but is a trait in another (Fig. 4b), or if two populations share a trait and differ only in its frequency of occurrence (Fig. 4c), or if an attribute is unique to one population but is not fixed (Fig. 4d), this attribute cannot distinguish phylogenetic species.

The character or characters that distinguish two phylogenetic species must differentiate every comparable individual of each species from every individual of the other. If species were recognized on the basis of trait differences alone (Figs. 4b–d), it would be possible for identical individuals to occur in two different species; the two species then could not be diagnosed. If parsimony (however inappropriately) is applied to two identical individuals, they must be interpreted as more closely related to each other than to any individuals from which they differ. Thus, such species would be "polyphyletic." In light of the requirement of diagnosability, recall our comments concerning age-specific and gender-specific variants, and that the occurrence of an original and one or more transformed states of a character does not negate the historical fixation of that character. Phylogenetic species are diagnosable because their differences are fixed, and barring future gene flow between species they will remain diagnosable because they cannot evolve to identity in the absence of parallel (i.e., nonhomologous) mutation events.

Although diagnosability is most easily described in terms of character constancy in one species versus absence of the character in another (as in most examples we cite), this condition is not necessary for two species to be distinct. One phylogenetic species may carry alleles A and C at a particular locus, whereas another carries B and D. Although neither is genetically fixed, the two are diagnosable, and the second condition of Hennig's model holds, as long as A/C is regarded as a single character and B/D as another. This is consistent with the perspective that loss of the plesiomorphic character state is actually the significant event in speciation (Nixon and Wheeler, 1992).

In terms of population-level processes, phylogenetic species are the least inclusive populations or sets of populations among which there is character-based evidence, in the form of fixed differences, that gene exchange does not occur. We do not argue that errors in observation and interpretation cannot occur (see below) but that character evidence invoked in support of hypothesized species boundaries should take the form of fixed differences, not frequency differences.

Although the PSC makes no reference to potential for gene flow, the criteria do imply an actual absence of interbreeding between any pair of phylogenetic species. Given two phylogenetic species, the fixed differences that differentiate them provide evidence of divergence, that is, that they are evolutionarily independent. On this basis, the PSC resembles the lineage concept embodied in the evolutionary species concept of Simpson (1961; see also Wiley, 1978, 1981; Frost and Hillis, 1990). However, the "historical fate" (i.e., future) of a

species (Wiley, 1981:25) is not relevant to the status of phylogenetic species as units of a phylogenetic descent system. Character differences are evidence that gene flow has ceased between phylogenetic species, but what has prevented gene flow is a separate question. Character divergence is not evidence of lost potential for genetic interaction between phylogenetic species; it is evidence for a historical absence of gene exchange. Intrinsic reproductive isolating mechanisms (such as mating behavior or physiological pollen–stigma incompatibilities), if they are indeed fixed differences, are legitimate characters for the demarcation of phylogenetic species, but this class of characters holds no special position except in suggesting stability of the observed situation.

We have argued that phylogenetic species are the least inclusive units for which there is discoverable evidence of conformity with Hennig's model and that these entities are potentially appropriate terminals for phylogenetic analysis. However, phylogenetic species are not monophyletic. This is because, by definition, discoverable hierarchic relationships do not exist below the level of phylogenetic species. If a hierarchic substructure were to be discovered within a phylogenetic species, in the form of distinct and constant character differences among populations or groups of populations, further subdivision along these lines would be called for. Because less inclusive units than a phylogenetic species cannot be the subject of phylogenetic analysis, subunits within a species cannot be demonstrated to be related to each other hierarchically in a manner that would justify use of the term "monophyletic" for the species itself.

A related reason for rejecting the term "monophyletic" (also "paraphyletic") with reference to individual phylogenetic species derives from the notion of most recent common ancestor. In a hierarchic descent system, any specified set of terminals has a single MRCA. When cladistic sister species differ only in the possession by one of them of a single autapomorphic character, there is evidence that divergence has occurred but not that the MRCA of all individuals (or populations) in either of the species lacks descendants in the other (Fig. 3). Indeed, the very meaning of MRCA for individuals in sexual populations is ambiguous because each "ancestor" is a mating pair or a self-fertilizing individual, and because an individual can mate with any number of other individuals, including its own descendants. Even when this ambiguity is set aside, one is still confronted with the difficulty of asserting that all individuals within one species share a common ancestor that is not also an ancestor of individuals within another species. Although individuals (in the same or in different species) that share a specified attribute are descendants of a common ancestral individual that first bore the attribute, the absence of a specified attribute in an individual does not indicate that it is not a descendant of that ancestor (Figs. 2, 3). In contrast, a character (not trait) shared by two or more species within a hierarchic descent system is evidence that these species are descendants of a common ancestral species that also bore this character, whereas the absence of the character from another species is evidence that this species is not a descendant of that ancestor.

Populations

Until this point, we have used the term "population" in a general sense. This usage has been sufficient because however broadly or narrowly one might circumscribe individual populations, it has been sufficient to regard them as the arenas in which sexual reproduction, genetic recombination, and character fixation occur. Because phylogenetic species are discoverable by means of characters, each of which arises initially in an individual, members of a phylogenetic species are historically related. Furthermore, the attributes that eventually become characters do so by the population processes we have just mentioned (e.g., recombination). Thus, if every phylogenetic species exhibits a unique combination of characters, each is an extended genealogical population, all of whose constituent individuals are historically related. Our continued use of "pop-

ulation" in the context of characters and traits of phylogenetic species therefore refers to a genealogical unit that, if marked by a unique character combination, is equivalent to a phylogenetic species.

Another less inclusive population concept also is pertinent. Although phylogenetic species can be equated with extended genealogical "populations," a phylogenetic species may comprise more than one "population"; in this usage we refer to local genealogical units, the arenas in which most genetic recombination occurs. No ideal distinction can be drawn between local populations, whose constituent individuals are in more immediate reproductive contact with each other, and the extended genealogical populations that are phylogenetic species. But unless a complete pedigree of all sexual organisms becomes available, empirical delimitation of the widespread population systems we call phylogenetic species will remain a matter of analyzing distribution patterns of observable attributes within and among local populations.

Given the goals of phylogenetic analysis, and the PSC as an arbiter of the appropriate units of analysis, how can putative phylogenetic species be delimited empirically? If all genetic variation that actually exists among living things were trait variation (not character variation), there would be just one recognizable phylogenetic species: a polymorphic aggregate of populations that could not be subdivided (Nixon and Wheeler, 1990). Thus, the empirical delimitation of phylogenetic species amounts to the partitioning of attribute variation into characters (fixed differences among species) and traits (variation among individuals within species), and the local population is the arena in which the relevant observations can be made. This inference, that an attribute is a populational polymorphism (i.e., an inference concerning the attribute's distribution), allows systematists to assign nonidentical individuals to the same species. Botanists regard white-flowered and blue-flowered individuals in a population of forget-me-nots as conspecific precisely because the variant attributes of these individuals are perceived (correctly or not) as intrapopulational polymorphisms.

Population Aggregation Analysis: Delimitation of Phylogenetic Species on the Basis of Population Polymorphism

Population aggregation analysis is a method for the identification of phylogenetic species. This method is designed to distinguish traits from characters on the basis of variation patterns observed within local populations. The basic principles are as follows. All individuals of a local population are regarded as belonging to the same phylogenetic species. If identical individuals can be drawn from two local populations (i.e., if no character distinguishes the two populations), the two populations belong to the same species. On the basis of these two operational principles, phylogenetic species are delimited by successive rounds of aggregation (grouping) of local populations that are not distinct. Profiles of attribute occurrence among individuals in local populations first are assembled, then populations that are not distinct are grouped until all remaining populations or population groups are distinct. The basic data set therefore consists of attribute scores (presence/absence) for population samples of individuals. The data table is a familiar individual-by-attribute matrix, with individuals apportioned among populations (Table 1). Each population can include any number of individuals, and this number can vary among populations within the analysis.

The initial assumptions that determine the outcome of the analysis are the assignment of attribute scores to individuals and the assignment of each individual to a local population. If one or a small number of populations affect the results profoundly, these populations are easily identified. The discovery that a few populations blur an otherwise distinct species boundary does not mean that the analysis has been conducted improperly; populations that are polymorphic for attributes that otherwise

TABLE 1. Presence/absence of 10 attributes among five individuals from each of two hypothetical populations, and summary population profiles. The populations are distinct on the basis of attribute 9 (see text); all other attributes are fixed in both populations (e.g., no. 1) or present but not fixed in one or both populations (e.g., nos. 2, 4, 5).

Population / Individual	Attribute[a]									
	1	2	3	4	5	6	7	8	9	10
Population 1										
1	1	0	1	1	0	0	0	1	0	1
2	1	1	1	1	1	0	0	1	0	0
3	1	1	1	1	0	0	0	1	0	1
4	1	0	1	1	0	0	0	1	0	0
5	1	1	1	0	0	0	0	1	0	1
Population profile	1	±	1	±	±	0	0	1	0	±
Population 2										
1	1	0	1	1	1	1	0	1	1	1
2	1	0	1	1	0	0	1	1	1	1
3	1	0	1	1	1	0	0	1	1	1
4	1	0	1	1	1	0	1	1	1	1
5	1	0	1	1	1	0	0	1	1	1
Population profile	1	0	1	1	±	±	±	1	1	1

[a] 0 = absent; 1 = present (in individual) or fixed (in population); ± = present but not fixed in population.

delimit species may represent regions of primary intergradation (clinal variation) or incomplete divergence or zones of hybridization between once-distinct species.

The analysis proceeds initially by summarizing profiles of individuals from each population as a population profile that reflects the fixed presence, absence, or inconstant presence (populational variation) of each attribute (Table 1). Populations then are compared with each other sequentially to determine whether any are distinguishable, on the basis of at least one attribute that is fixed in one and absent from the other. When two populations are distinct, they become the nuclei of separate species. If a fixed difference does not exist between two populations, they are interpreted as conspecific, and an aggregate variation profile reflecting this multipopulation species is generated (Table 2). This profile reflects each attribute as present and fixed (if present and fixed in all constituent populations), absent (if absent from all constituent populations), or present but not fixed (if present but not fixed in at least one

TABLE 2. Population profiles (see Table 1) representing absence, fixed presence, and nonfixed presence of 10 attributes among five hypothetical populations, and summary species profiles produced by aggregation of nondistinct populations. Species I and II are distinct on the basis of attribute 9 (see text); all other attributes are fixed in both species (e.g., no. 3) or present but not fixed in one or both species (e.g., nos. 1, 2, 10).

	Attribute[a]									
	1	2	3	4	5	6	7	8	9	10
Population 1	1	±	1	±	±	0	0	1	0	±
Population 3	±	±	1	0	±	0	±	1	0	0
Population 4	±	1	1	0	±	0	±	1	0	0
Population 5	0	±	1	0	±	0	0	±	0	0
Species I	±	±	1	±	±	0	±	±	0	±
Population 2	1	0	1	1	±	±	±	1	1	1
Population 6	1	0	1	1	0	1	±	±	1	1
Population 7	1	0	1	1	0	1	±	±	1	±
Species II	1	0	1	1	±	±	±	±	1	±

[a] 0 = absent; 1 = fixed; ± = present but not fixed.

constituent population) in the species. Following each episode of aggregation, pairwise comparisons are made between the newly generated multipopulation species profile and the profiles of all previously generated species plus those of populations that have not yet been aggregated, and further aggregation proceeds as warranted.

The analysis terminates when all populations have been introduced into the analysis and further aggregation is unwarranted (Table 2). At this point, all populations have become aggregated into a single species, or they have been apportioned among two or more distinct species. Because an attribute that is inconstant in any one population of a multipopulation group is inconstant for the group as a whole, two populations may be distinct from each other and yet be assigned to the same species. This situation occurs when an attribute is constant in one population, absent from a second, and present but not fixed in a third (e.g., Table 2, attribute 1 in species I) and suggests the presence of clinal variation or hybridization between once-distinct species.

Although the procedure is iterative, the results of the analysis are independent of the sequence in which populations are introduced. Because variation for an attribute within any population renders that attribute inconstant within any group (species) to which the population eventually is assigned, each step of aggregation (e.g., the assignment of a population to a previously assembled group) either increases or leaves unchanged the number of attributes that are variable for the resulting group. Hence, for any given data set, two populations that are not distinct from each other always are assigned to the same species. Also, when two populations are distinct, but their differences are bridged by polymorphisms in other populations, the two eventually will become aggregated, regardless of the sequence in which populations are introduced.

Error

For any particular data set, the analytical procedure we have described generates a single result. The result is the identification of a number of putative phylogenetic species, each comprising one or more populations. The primary elements that lead to this result are the hypotheses of homology that underlie each attribute score and the assignments of individuals to populations. Any of these hypotheses may be incorrect; various forms of error in these basic hypotheses influence the results of the analysis.

Incorrect homology assessment.—Incorrect assessment of homology is a familiar problem in phylogenetics, and it can affect the results of population aggregation analysis as well. One aspect of this problem is the failure to recognize a transformed state of a character as such. For example, a population may be polymorphic at a given locus for alleles A and B. If B is a transformed state of A but is not recognized as such, the population is not interpreted as (historically) fixed for character A.

Analyses of isozyme data from the grass genus *Puccinellia* (Davis and Manos, 1991; Davis and Goldman, 1993; Davis, unpubl. data) have detected rare alleles that occur in just one or a few populations of a single species (as delimited by other characters). The presence of the rare alleles renders the nearly fixed alternative alleles apparently inconstant in occurrence. If, on the basis of a gene tree, all alleles at a locus in a population or population system are explicitly hypothesized to represent a character (i.e., an original state and an exclusive set of transformed states), historical character fixation can be inferred.

Undersampling of attributes.—Undersampling of attributes (i.e., too few loci examined) reduces one's ability to resolve differences between actual phylogenetic species because every unsampled attribute is a potential species-delimiting character. Conversely, under the terms of this analytical procedure a newly examined attribute cannot negate interspecific boundaries that already have been determined on the basis of previously examined attributes, although newly observed attributes can provide evidence that favors further division of species. Thus, undersampling of attributes consistently biases results to-

wards the recognition of fewer species than actually exist.

Undersampling of individuals within populations.—If few enough individuals are examined within a population, an actual trait might appear to be either fixed in it or absent from it. The examination of additional individuals cannot negate polymorphisms already discovered, but it can reveal additional polymorphisms. Thus, undersampling of individuals within populations biases the results of an analysis toward the recognition of more species than actually exist and toward false resolution of hierarchic structure (Nixon and Wheeler, 1992).

Undersampling of populations.—When a previously unsampled species is brought into an analysis, the number of known species grows. However, the inclusion of additional populations in a study also can cause the perceived number of species to drop, as polymorphic populations cause otherwise distinct populations to become aggregated into a single polymorphic species. The history of taxonomic discovery reflects both of these effects of increased sampling. As the world's biota becomes better sampled, new species are discovered; simultaneously, intermediate individuals provide evidence that previously delimited species should be combined. The bias of undersampling in any particular case (i.e., in a given multispecies study group) depends upon the relative rates at which three sorts of populations are discovered: (1) the first population sampled of each actual species; (2) additional and apparently distinct populations of species already discovered; and (3) populations that bridge differences between distinct conspecific populations previously interpreted as separate species.

Incorrect delimitation of populations.—The limits of biological populations are difficult to assess. If one local population is incorrectly interpreted as two or more, the single species to which this population belongs may be misinterpreted as more than one phylogenetic species. This occurrence is not unlike the results obtained when individuals, rather than populations, are treated as the elements to be aggregated into species; traits, which vary within a species, are then misinterpreted as species-delimiting characters, and actual species are incorrectly subdivided.

Alternatively, individuals of two (or more) phylogenetic species might be interpreted incorrectly as elements of a single local population. In this case, actual species-delimiting characters are misinterpreted as inconstant traits of a single population and hence as traits of the single putative species to which that "population" is assigned.

Attributes that are simultaneously constant in one or more populations within a species, absent from one or more other populations, and present but inconstant within a third set of populations are specifically identifiable among the results of an analysis (Table 2). The discovery of all three of these conditions among the populations of a species signifies one of two things. First, the observed pattern may legitimately reflect existing patterns of intraspecific variation. A phylogenetic species that exhibits fixation of different alleles at a locus in some populations and polymorphism in others does include populations that might have been interpreted as separate species if the populations that exhibit polymorphism had not been discovered. This condition might indicate that the population system includes two or more incipient species that are not yet completely distinct or that once-distinct species have subsequently hybridized. In either of these cases, however, the analysis has correctly identified departure from hierarchy.

A second possible cause of such a pattern is that two species exist, but the boundary between them has been obscured by error in population delimitation. In this case, each of the apparently polymorphic local populations actually comprises two or more locally sympatric but genealogically independent populations that represent different species.

Parallel fixation.—Several populations that are polymorphic for the same trait may proceed independently to fixation for its presence, and others may lose the trait. When no polymorphic population remains, two phylogenetic species will ap-

pear to have been discovered. In this case, the populations that constitute each putative species do not share a unique history, although this is suggested by the character distribution.

Conclusions

The goals of systematic biology are to discover the various forms of life that exist and have existed, to reconstruct as far as possible the relationships among these forms, and to create a system of classification that reflects these relationships. If descent relationships are hierarchic above a certain level, phylogenetic relationships can be reconstructed at this level and above on the basis of a nested hierarchy of characters. Below this level, sexual and parasexual recombination generate nonhierarchic distributions of variable attributes (traits). This is the point at which hierarchic configurations of attribute variation, although they can be generated, no longer reliably reflect historical relationships. It is imperative that methods be developed for the identification of this crucial dividing line between hierarchic and reticulate descent relationships.

The phylogenetic species concept identifies diagnosability as a criterion for determining whether a particular grouping of organisms should be treated as a terminal for phylogenetic analysis. We have described a procedure for delimiting phylogenetic species on the basis of variant and invariant attributes observed within sexual populations. Few groups of organisms have yet been analyzed by this procedure, so it is difficult to predict whether its widespread application will result in the recognition of greater or lesser numbers of species than are conventionally recognized; breeding behavior, population size, dispersibility, and other factors probably will influence the number of species that are recognized. In groups in which species are currently recognized on the basis of trait differences alone, the number of recognized species should decline. Many currently recognized species may be more appropriately interpreted as racial variants or varieties, and levels of homoplasy often reported in analyses of closely related "species" may be attributable in part to the nonhierarchic descent relationships among these entities. In terms of one of the fundamental practices in systematics, species delimitation, our goal is to stimulate increased attention to population variation. The phylogenies that systematists propose are as dependent upon constancy of character distributions within terminals as they are upon hypotheses of character homologies.

Acknowledgments

We thank R. Bateman, A. Bruneau, D. Cannatella, W. DiMichele, J. Doyle, J. Freudenstein, M. Luckow, P. Manos, B. Mishler, R. Soreng, Q. Wheeler, and two anonymous reviewers for useful discussions of the ideas presented here and for comments on drafts of this paper.

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Received 10 February 1992; accepted 19 March 1992