MATHEMATICAL CONSEQUENCES OF THE GENEALOGICAL SPECIES CONCEPT

RICHARD R. HUDSON[1] AND JERRY A. COYNE[2]
Department of Ecology and Evolution, The University of Chicago, 1101 E. 57th Street, Chicago, Illinois 60637
[1]*E-mail: rr-hudson@uchicago.edu*
[2]*E-mail: j-coyne@uchicago.edu*

Abstract.—A genealogical species is defined as a basal group of organisms whose members are all more closely related to each other than they are to any organisms outside the group (''exclusivity''), and which contains no exclusive group within it. In practice, a pair of species is so defined when phylogenies of alleles from a sample of loci shows them to be reciprocally monophyletic at all or some specified fraction of the loci. We investigate the length of time it takes to attain this status when an ancestral population divides into two descendant populations of equal size with no gene exchange, and when genetic drift and mutation are the only evolutionary forces operating. The number of loci used has a substantial effect on the probability of observing reciprocal monophyly at different times after population separation, with very long times needed to observe complete reciprocal monophyly for a large number of loci. In contrast, the number of alleles sampled per locus has a relatively small effect on the probability of reciprocal monophyly. Because a single mitochondrial or chloroplast locus becomes reciprocally monophyletic much faster than does a single nuclear locus, it is not advisable to use mitochondrial and chloroplast DNA to recognize genealogical species for long periods after population divergence. Using a weaker criterion of assigning genealogical species status when more than 50% of sampled nuclear loci show reciprocal monophyly, genealogical species status depends much less on the number of sampled loci, and is attained at roughly 4–7 *N* generations after populations are isolated, where *N* is the historically effective population size of each descendant. If genealogical species status is defined as more than 95% of sampled nuclear loci showing reciprocal monophyly, this status is attained after roughly 9–12 *N* generations.

Key words.—Coalescence, genealogical species, phylogeny, reciprocal monophyly, species concept.

Many species concepts have been suggested within the last two decades (see Claridge et al. 1997; Ereshefsky 1992; Wheeler and Meier 2000). Most of these fall into two categories: concepts involving reproductive or phenotypic distinctness of populations, such as Mayr's biological species concept (BSC; Mayr 1963), and phylogenetic species concepts that resolve species using phylogenies based on molecular and/or phenotypic traits (Baum 1992). Among the latter, one of the most widely discussed concepts is the genealogical species concept (GSC), first proposed by Baum and Shaw (1995).

As defined by Shaw (1998, p. 48), ''A *genealogical species* is a basal, exclusive group of organisms, whose members are all more closely related to each other than they are to any organisms outside the group, and that contains no exclusive group within it.''

''Basal'' means that each species so defined does not contain subgroups, all of whose members are more closely related to each other than to individuals in other subgroups within the species. ''Exclusivity'' is explained within the definition, meaning that each individual in a genealogical species (GS) is more closely related to individuals belonging to the same GS than to organisms outside the GS. Exclusivity is generally specified genetically: ''A group of organisms is exclusive if their loci coalesce more recently within the group than between any member of the group and any organisms outside the group'' (Baum and Shaw 1995, p. 296). Avise and Ball (1990) were the first to consider monophyly as a method for historically grouping organisms, but did not deem it a good way to identify species.

In practice, two groups are recognized as distinct GSs when they are *reciprocally monophyletic*; that is, when phylogenetic analysis shows that, at each locus, alleles found within each GS are more closely related to each other than to alleles of the

same locus in the other GS. In this sense, monophyly does not mean that every allele at every sampled locus descends from a single multilocus genotype contained in a single ancestral individual. Rather, *for each sampled locus,* all alleles share a common ancestor within that group. Alleles for different loci are likely to descend from different individual organisms during the history of the group, but monophyly and exclusivity nevertheless apply to each locus. This is the way that we construe monophyly in the following discussion.

The criterion of *reciprocal* monophyly does not deal with the messy situation that can arise if a species descends from only one portion of another species, as when individuals from a single population of a widespread continental species invade an island. Genetic differentiation of the island population may result in its genetic exclusivity and status as a GS, but individuals in the mainland population from which the migrants were drawn might be more closely related to individuals in the island GS than to individuals in other mainland populations. In this case, the entire mainland species is no longer an exclusive group—it is paraphyletic with respect to the island population and loses its status as a GS at the moment that the island species becomes exclusive. As individuals in mainland populations are no longer members of *any* GS, some have suggested calling such groups ''metaspecies'' (de Queiroz and Donoghue 1988).

Although in principle a recognition of GS status should be based on assessing relatedness from a large sample of loci (after all, it is individual organisms and not genes that are members of a GS), in practice the recognition of reciprocal monophyly must be made using a limited number of loci and alleles.

One problem arising with the GSC is to specify what *proportion* of loci must be reciprocally monophyletic before a group is considered a GS. A demand for reciprocal monophyly of *all* loci is extreme, because forms of balancing se-

lection, such as heterosis or frequency-dependent selection, can keep alleles polymorphic for long periods of time. If for example, alleles A and B are maintained in an ancestor by balancing selection and this selection persists in two descendant taxa, an A allele in taxon 1 can be more closely related to an A allele in taxon 2 than to any other alleles in taxon 1. Thus the alleles of this locus do not coalesce within each taxon, and can remain in this condition long after most other loci have become reciprocally monophyletic. This is the situation at the MHC locus in humans versus chimps and in rats versus mice (both pairs diverged approximately 10 million years ago; Ayala and Escalante 1996; Figueroa et al. 1988), and for self-incompatibility alleles among plant species in the genus *Brassica* (Uyenoyama 1995).

Although advocates of the GSC have recognized the problems with demanding complete reciprocal monophyly—whose abandonment causes fuzzy boundaries for attaining GS status (e.g., Baum and Shaw 1995, p. 301)—they have not explicitly addressed the question of what *proportion* of surveyed loci must be reciprocally monophyletic to allow GS status. Shaw (2001), however, suggests that GS status might be recognized if *most* loci are reciprocally monophyletic. Of course judgments about GSC status using less than 100% reciprocal monophyly involve an arbitrary threshold, similar to identifying biological species when there is any gene flow between taxa.

Despite this arbitrariness—a problem which plagues most species concepts—the GSC seems a reasonable way to recognize species if one adheres to phylogenetic rather than to phenotypic or reproductive criteria; and the GSC has drawn approbation even from those who adhere to the biological species concept (e.g., Harrison 1998). However, despite Shaw's (1998, p. 48) note that ''an explicit mathematical treatment of the boundaries of genealogical groups (indeed of *any* natural group) is badly needed,'' there has been no general discussion of how long it will take for two isolated populations to achieve reciprocal monophyly at more than one gene. Tajima (1983), however, has calculated times to reciprocal monophyly for two autosomal alleles at a single locus, assuming two daughter populations derived from a common ancestor; Wakeley (2000) calculated times to reciprocal monophyly for subdivided populations and species, also using samples of two alleles at one locus; and Neigel and Avise (1986) give simulation results for reciprocal monophyly of a single mitochondrial locus.

Here we present a general mathematical analysis of genealogical speciation based on the coalescent theory for the fixation of neutral alleles. Using this theory, we give, for various numbers of sampled loci and alleles, estimates of the probability of observing reciprocal monophyly for different divergence times, population sizes, and criteria for genealogical species status. Although we are advocates of the BSC (and, at the end, give some reasons why we favor it over the GSC), our main purpose is not to argue for the validity of one or another species concept. Rather, our goal is to estimate how long it will take genealogical speciation to occur when species are identified using loci that evolve neutrally.

METHODS AND MATERIALS

To calculate the probability of reciprocal monophyly of samples drawn from two populations we make the following assumptions:

(1) Two diploid populations were derived by random division of an ancestral population t_s generations ago and have been genetically isolated since that time.

(2) Effective population size, *N*, remains constant in both descendant populations. The size of the ancestral population is irrelevant so long as it is substantially larger than the number of alleles sampled at any locus.

(3) There is no selection at the locus of interest or any linked loci.

(4) There is no recombination *within* the locus of interest.

(5) The derived populations are based on a random division of a panmictic ancestral population and are themselves panmictic.

(6) Gene trees are correctly estimated. (That is, we study the properties of the actual gene trees, whereas in practice one must used estimated trees, which are subject to additional stochastic errors.)

(7) For results concerning multiple loci, we assume statistical independence of the gene trees at different loci. This is a very good approximation for loosely linked loci unless the population size is very small.

In the following discussion we use ''allele'' to mean ''gene copy'' rather than ''a distinguishable genetic variant.'' In this terminology, different alleles are simply different copies of a gene and do not necessarily have different sequences.

We consider the case in which a sample size of *k* alleles from a single locus is taken from each population. In this case the probability of reciprocal monophyly of the samples is given by:

$$P_{rm}(t_s) = \sum_{i=1}^{k} \sum_{j=1}^{k} C(i, j) g_{ki}(t_s) g_{kj}(t_s), \qquad (1)$$

where $g_{ki}(t)$ is the probability that *k* sampled alleles from one population have *i* distinct ancestors *t* time units in the past and where $C(i, j)$ is the probability that a sample of size $i + j$ from one population has a genealogy such that a specified subsample of *i* alleles and the rest of the sample (of size *j* alleles) are reciprocally monophyletic. Equation (1) is obtained by considering the number of distinct ancestral lineages from each population that exist at time t_s. In fact, the summand in equation (1) is the probability that there are *i* distinct ancestors of the sample from one population and *j* distinct ancestral lineages from the other population at time t_s, and that these $i + j$ lineages randomly coalesce in such a way that the samples are reciprocally monophyletic. The probabilities $g_{ki}(t)$ are given by equation 6.1 of Tavaré (1984). The probability $C(i, j)$ satisfies the recursion:

$$C(i, j) = \frac{\binom{i}{2}C(i - 1, j) + \binom{j}{2}C(i, j - 1)}{\binom{i + j}{2}}, \qquad (2)$$

where $C(1, 1)$ is one, and

$$\binom{j}{2} = j(j - 1)/2.$$

This recursion for $C(i, j)$ follows from considering the first coalescent event back in time, and the fact that under the neutral model all of the $(i + j)(i + j - 1)/2$ possible pairs of lineages are equally likely to coalesce. Unfortunately, Tavaré's formula for $g_{ki}(t)$ is a summation with terms of alternating signs, which can be difficult to calculate with adequate precision. To circumvent this difficulty we have estimated $P_{rm}(t)$ by standard coalescent simulations (Hudson 1990). This was done as follows. A large number of independent pairs of gene trees are generated for samples of size n. For each pair of trees, we note a_1 and a_2, the numbers of ancestors of each sample at time t_s, and calculate $C(a_1, a_2)$. The average of these $C(a_1, a_2)$ over many pairs of trees constitutes our estimate of $P_{rm}(t)$ and will be denoted $\langle P_{rm}(t)\rangle$.

The estimated probability of reciprocal monophyly at n loci is simply $\langle P_{rm}(t)\rangle^n$. The estimates of $P_{rm}(t)$ shown in Figures 1, 2, and 5 were obtained in this way, with 100,000 pairs of trees generated for each of a series of t_s values. The values given in Table 1 are interpolated from these same simulation results. The mitochondrial results are obtained by simply adjusting the t_s value, assuming that the effective population size of the mitochondrion is one quarter that of a nuclear locus. (One could also use chloroplast DNA [cpDNA] instead of mitochondrial DNA because both are haploid and in most cases inherited uniparentally.)

We also calculated the probability of reciprocal monophyly when one considers genealogical speciation to have occurred when more than either 50% or 95% of sampled loci are reciprocally monophyletic. To obtain the curves in Figure 3 that deal with these thresholds, our estimates of the probability of reciprocal monophyly, $\langle P_{rm}(t)\rangle$ described above and plotted in Figure 1, were used as the probability parameter of a binomial distribution. For example, to calculate the probability of more than 50% of loci showing reciprocal monophyly, for 15 loci, we calculate:

$$\sum_{j=8}^{15} \binom{15}{j}\langle P_{rm}(t)\rangle^j (1 - \langle P_{rm}(t)\rangle)^{15-j}. \tag{3}$$

To calculate the probability of more than 95% of 25 loci showing reciprocal monophyly, we calculate:

$$\sum_{j=24}^{25} \binom{25}{j}\langle P_{rm}(t)\rangle^j (1 - \langle P_{rm}(t)\rangle)^{25-j}. \tag{4}$$

Estimates of the probability of reciprocal monophyly using these relaxed criteria are given in Figure 4.

We also considered the probability that, when samples are drawn from each of two populations, the first sample is monophyletic but the second sample may be either monophyletic or paraphyletic relative to the first sample. This gives us an idea of the probability of determining GS status for only a single descendant population. To estimate this probability, denoted $P_m(t)$, we use the same method used to estimate $P_{rm}(t)$, except that $C(i, j)$ is replaced by $C^*(i, j)$, which satisfies the same recursion as $C(i, j)$ with the different boundary condition: $C^*(1, j) = 1$, for all j. The estimates of $P_m(t)$ shown in Figures 4 and 5 were obtained in this way.

RESULTS

Figure 1 shows, for different numbers of loci (with 100 alleles sampled at each locus), the probability that, at a given time after separation, two isolated populations will be reciprocally monophyletic at all sampled loci. Time is given in units of N generations, where N is the effective size of each population. Table 1 gives the times since population separation at which samples of different numbers of loci will be reciprocally monophyletic with three probabilities of interest: 0.05, 0.50, and 0.95. As the time to monophyly is measured in units of N generations, it is obvious that the time to GS status is directly proportional to population size. The curve for $n = 1$ locus differs somewhat from that of Tajima (1983): ours shows a slower approach to reciprocal monophyly because Tajima considers a sample of only two alleles from each population.

We have also included in Figure 1 the times corresponding to observing complete reciprocal monophyly (i.e., exclusivity for all genes) for an entire genome of a representative eukaryote, *Drosophila melanogaster*. Although *D. melanogaster* has roughly 14,000 loci (Rubin et al. 2000), the number of effectively independent genealogical units, (IGUs; i.e., the number of genomic segments whose passage to monophyly is nearly independent of that for all other segments), is less than the number of loci. Under our neutral model, the statistical dependence between two loci depends on the parameter $4Nc$, where c is the per generation recombination rate between the two loci. The expected value of r^2, a measure of linkage disequilibrium, is approximately $1/4Nc$ for large values of $4Nc$ (Ohta and Kimura 1968). To obtain an approximate number of IGUs we assume that sites separated by $4Nc = 1000$ are independent units, because r^2 will be only 0.001 for such pairs of sites. Given the map length of the *Drosophila melanogaster* genome as 287 cM, and assuming that N for *D. melanogaster* is 10^6 (Kreitman 1983), we find the number of IGUs to be about 11,500 ($\approx 287 \times 10^{-2} \times 4 \times 10^6/1000$).

We have not presented the time to reciprocal monophyly for descendant populations of unequal size. However, when the disparity between these sizes is very large, as when a widespread continental species colonizes an island, monophyly in the small island population (and hence its recognition as a genealogical species) will occur very quickly relative to the set of mainland populations, which remains a metaspecies for a long time thereafter. In such a case, the time to reciprocal monophyly will be roughly equal to the time needed for the larger population to become monophyletic. (We present below calculations for the probability of monophyly for an individual taxon).

All of the curves shown in Figure 1 are derived from the basic curve for one autosomal locus. Because the effective population size of a mitochondrial (or chloroplast) locus is one-quarter that of an autosomal locus, the mitochondrial DNA (mtDNA) curve is derived from the autosomal curve by shifting the horizontal position of each point from t to $t/4$. (The curve shown for mtDNA in Fig. 1 is similar to that generated by Neigel and Avise [1986].) Because we assume that loci are independent, the curves involving several nuclear loci are derived simply by taking powers of the probability

TABLE 1. Times (given in units of N generations, where N is the effective population size of each descendant taxon) corresponding to different probabilities of reciprocal monophyly for two populations (left side) and monophyly for a single population (right side). These numbers are simply interpolations from the curves given in Figures 1 and 2. Calculations are given for different numbers of loci, assuming that 100 alleles are sampled for each locus. "11,500 nuclear loci" corresponds to the number of independent genealogical units of the *Drosophila melanogaster* genome (see text); for this number of loci we were unable to calculate the time corresponding to probabilities of reciprocal monophyly of 0.95.

Number and location of loci	Probability reciprocal monophyly			Probability monophyly		
	0.05	0.50	0.95	0.05	0.50	0.95
1 mitochondrial	0.38	0.94	2.20	0.29	0.71	1.80
1 nuclear	1.50	3.80	8.70	1.20	2.80	7.30
5 nuclear	4.00	6.70	11.80	3.00	5.50	10.50
15 nuclear	6.00	8.90	14.10	4.80	7.60	12.80
25 nuclear	7.00	9.90	15.20	5.70	8.60	13.90
11,500 nuclear	19.10	22.1	—	17.90	20.80	26.30

curves for one locus. For example, if at a given time the probability of a observing reciprocal monophyly for a single locus is p, then the probability of observing reciprocal monophyly for every locus in a sample of five at that time is p^5.

Obviously, as more loci are added to the sample, the probability of observing reciprocal monophyly of all loci at any time decreases; and the probability of changing the GS status of two taxa by adding a given number of loci to a sample of S loci decreases as S becomes larger. The curves in Figure 1 do not approach a limit as the number of loci approaches infinity, so reciprocal monophyly is never attained for a genome having an infinite number of IGUs.

Presumably, one often uses a sample of loci to infer the GS status of the entire genome. If this is the goal, several conclusions follow. First, if one uses more than a handful of loci, it takes a reasonably long time (if population size is large) to observe a high probability of reciprocal monophyly. For a sample of 25 nuclear loci, for example, reciprocal monophyly is reached with 95% probability after 15.2 N generations (Table 1). Second, unless taxa have been genetically isolated for a very long time, it is inadvisable to use monophyly for a single mitochondrial or chloroplast locus to diagnose one or more genealogical species. Because of the smaller number of copies of each mitochondrial and chloroplast locus than of each autosomal locus, mtDNA and cpDNA will exhibit reciprocal monophyly with high probability well before such monophyly is observed at even a small number of nuclear loci. Moreover, because genes in either a mitochondrion or a chloroplast are completely linked, no additional information about monophyly is gained from using more than one gene of reasonable size in these organelles: when a single such locus has become monophyletic, all other loci in the organelle are also monophyletic.

The time course for increasing probability of reciprocal monophyly of a single locus (and therefore for more loci) does not depend heavily on the number of alleles sampled at that locus (Fig. 2), and for longer divergence time there is virtually no effect of allele number on GS status. (Although curves are shown for only five and 100 alleles sampled per gene, the curve for 400 alleles [not shown] is nearly coin-
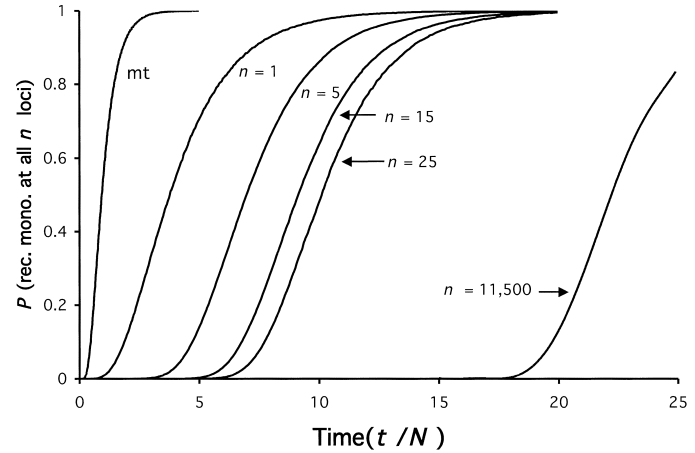

FIG. 1. Probabilities of observing reciprocal monophyly with time for populations that are genetically isolated. Curves are shown for a single mitochondrial locus and for samples of different numbers of nuclear loci. Time is measured in units of N generations, where N is the effective population size of each of the two descendant populations. mt, mitochondrial DNA, n, number of nuclear loci sampled. We assume that 100 alleles are sampled for each locus.

cident with that using 100 alleles.) Thus, if effort is limited, it is far more efficient to assess GS status by increasing the number of loci than by increasing the number of alleles sampled per locus.

What if one relaxes the criterion for GSC status by demanding reciprocal monophyly at only a fraction of sampled loci? Figure 3 shows the probability curves for two such criteria: each species monophyletic for 50% or more of sampled loci (as suggested in Shaw 2002), and for 95% or more of sampled loci. (The latter criterion could be used only for our sample of 25 loci.) Using these less stringent criteria, one finds that the number of loci sampled has a much smaller effect on the probability of observing reciprocal monophyly than when one uses a strict criterion of 100% monophyly. This is because the probability of attaining success on every trial (corresponding to reciprocal monophyly of every sampled locus) is higher if one performs fewer trials as opposed
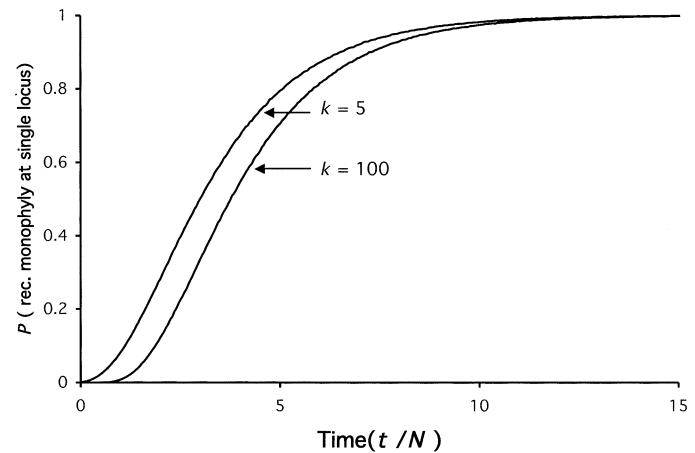

FIG. 2. Probabilities of observing reciprocal monophyly with time (measured in units of N generations) for a single locus, with two different sample sizes of alleles (k = 5 and k = 100).
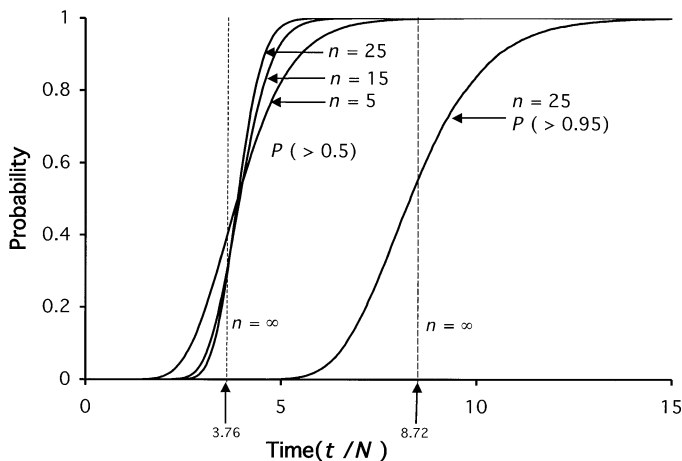
FIG. 3. Probability of observing reciprocal monophyly with time for two populations, when ''monophyly'' is defined as reciprocal monophyly at greater than 50% of the loci sampled in each population, $P > 0.5$; and when it is defined as reciprocal monophyly at greater than 95% of the loci sampled in each population, $P > 0.95$. n, total number of loci sampled in each population; 100 alleles are sampled for each locus.
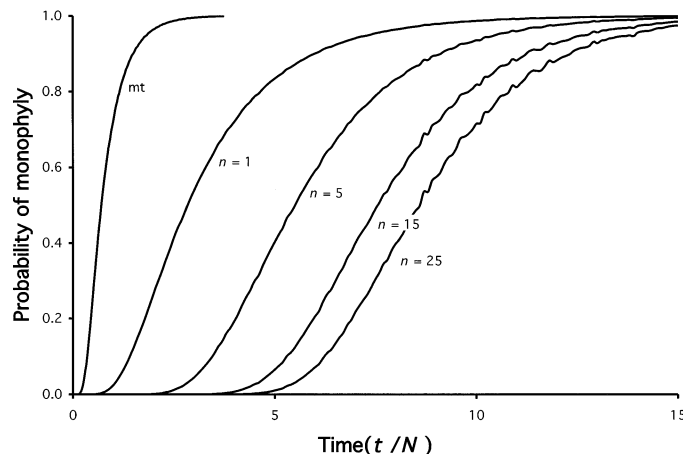

FIG. 4. Probabilities of observing monophyly with time for a single genetically isolated population. Curves are shown for a single mitochondrial locus and for samples of different numbers of nuclear loci. Time is measured in units of N generations, where N is the effective population size of each of the two descendant populations. mt, mitochondrial DNA, n, number of nuclear loci sampled. We assume that 100 alleles are sampled for each locus. The non-smooth character of the curve for large times for $n = 15$ and $n = 25$ reflect the stochastic error in our estimation of the probabilities in these cases.

to the larger number of trials corresponding to the more stringent criterion for monophyly. If one uses the ''greater than 50%'' criterion for GS status, all samples have a 50% probability of attaining this status after about 3.76 N generations, the time at which two separated populations become completely reciprocally monophyletic for a single locus with probability 50% (Table 1). Likewise, under the ''greater than 95%'' criterion, all samples have a 50% probability of attaining GS status after about 8.72 N generations, the time at which populations become completely reciprocally monophyletic at a single locus with probability of 0.95.

For a very large number of loci, roughly an entire genome, the transition from non-GS to GS status under the criterion of more than 50% reciprocal monophyly occurs very rapidly at 3.76 N generations; when the criterion of more than 95% reciprocal monophyly is used, GS status is attained at 8.72 N generations. The rapidity of these transitions reflects the fact that in a collection of many genes, there is almost no variation in the time at which more than half of them (or more than 95% of them) become reciprocally monophyletic, and these times are the same as those at which a single locus has a 50% (or 95%) chance of being reciprocally monophyletic. Under these relaxed criteria, one could predict when genealogical speciation would be observed for neutral loci with 100% probability if one knew the historical effective population size. However, one usually has little information about N. Because estimates are likely to be unreliable, GS status is best ascertained using actual genetic data. For a reasonable number of loci, a high probability of attaining GS status takes approximately 4–7 N generations using the ''greater than 50%'' criterion for reciprocal monophyly, and 9–12 N generations using the ''greater than 95%'' criterion for reciprocal monophyly. (A sample of at least 20 loci is required to use the latter criterion.)

Figure 4 shows the relationship between the time of genetic isolation of a single population from its ancestor and the probability of observing complete monophyly in a sample of

loci. Here we are concerned only with the GS status of one group and are not worried about whether the other descendant population is a metaspecies. The divergence times corresponding to probabilities of 0.05, 0.5, and 0.95 are given on the right side of Table 1. For a given number of loci, the time needed to observe monophyly with a given probability does not differ substantially whether one considers only one descendant or both descendant populations: typically, the time corresponding to a given probability of reciprocal monophyly is only 15–30% longer than for monophyly of a single population.

Figure 5 compares, for a sample of 100 alleles at one locus, the probability curve for reciprocal monophyly of two pop-
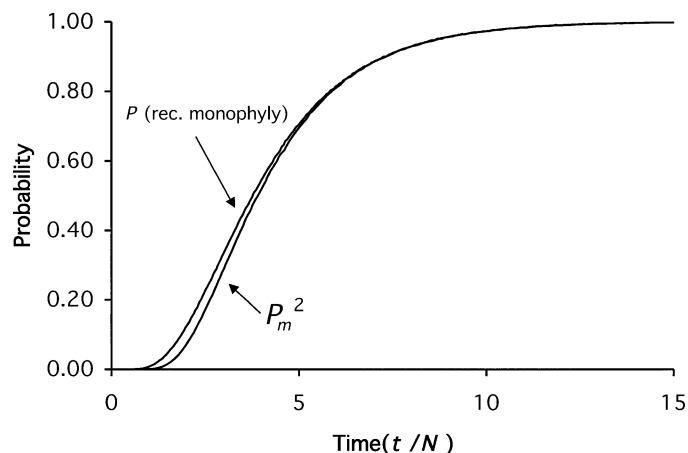

FIG. 5. The probability of observing reciprocal monophyly of two populations, P (rec. monophyly), compared to the square of the probability of observing monophyly in a *single* population (P_m^2). Curves are for a sample of 100 alleles from a single autosomal locus in each population.

ulations (the ''$n = 1$'' curve in Fig. 1) with the curve for the *square* of the probability of monophyly for a *single* population (the $n = 1$ curve in Fig. 5). The near-coincidence of these two curves shows that, at any time after genetic separation of populations, the probability of reciprocal monophyly in two populations of equal size is well approximated by the square of the probability of monophyly for a single population of that size. This enables one to extrapolate calculations for one taxon to two descendant taxa.

An additional species concept—the autapomorphic species concept (ASC) can also be addressed by our calculations. According to this concept, an autapomorphic species (AS) can be recognized if it is monophyletic for only a single derived trait (autapomorphy) that diagnoses the group (de Queiroz and Donoghue 1990; Nixon and Wheeler 1990), even if no other trait shows monophyly. Such traits can be phenotypic characters or genes. Among applications of the ASC are Young and Crother's (2001) diagnosis of a new species in the frog genus *Rana* because it is fixed for a derived allele at a single allozyme locus, and Leaché and Reeder's (2002) diagnosis of four species within the lizard *Sceloporus undulatus* based solely on mtDNA haplotypes.

We can use our calculations to determine the probability that at least one locus is diagnostic and either monophyletic or reciprocally monophyletic in a collection of neutrally-evolving loci in a pair of descendant taxa. For example, if at time t the probability of a locus being reciprocally monophyletic is p, the probability that, in a collection of n loci, none are reciprocally monophyletic is $(1 - p)^n$. Therefore, the probability that at least one locus in each taxon is reciprocally monophyletic (that is, that the taxa are both ASs) is $1 - (1 - p)^n$. We can use the curves in Figures 1 and 5 and the calculations in Table 1 to determine the probability of attaining AS status at a given time. For example, after $1.5 N$ generations, the probability that a single nuclear locus is reciprocally monophyletic in two taxa is 0.05 (Table 1). Therefore, the probability that in a collection of 25 loci, at least one is reciprocally monophyletic, is $1 - (1 - 0.05)^{25}$ or 0.723. Thus, although the probability of two descendant taxa both being recognized as GSs using one locus is only 5%, the probability of finding at least one reciprocally monophyletic locus out of a collection of 25 (and thus having the taxa recognized as ASs) is 72.3%. Similarly, whereas the probability of observing reciprocal monophyly for a single locus after $8.7 N$ generations is 0.95, the probability of observing reciprocal monophyly for at least one out of 25 loci is 0.99999997. Obviously, autapomorphic species status is attained much sooner than is genealogical species status, and this disparity increases with the number of genes (or traits) surveyed.

DISCUSSION

Because our model is one of pure neutral alleles whose substitution occurs by drift, the results given above will not be the same as those obtained when the sampled loci are either directly affected by selection or are neutral but closely linked to selected sites. Although the rate of substitution of neutral alleles—the molecular clock—is insensitive to population structure and selection at linked loci (Birky and Walsh

1988), this is not true for the pattern of genealogies and thus for the probabilities of monophyly necessary to define a GS. Balancing selection will slow down the coalescence of phylogenies and thus delay formation of a GS, while directional selection will speed up the formation of GSs. Because directional selection is probably more common than balancing selection, it is likely that for most loci the times to attain single-population or reciprocal monophyly will be shorter than the times given here, unless selection is counterbalanced by any retarding effects of population structure (see below). How selection affects the overall time to attaining GS status will depend on the number of loci sampled, the proportion of loci subject to balancing or directional selection, one's criterion for GS status, and other factors (see Shaw 2001).

We have not simulated the effect of population structure on the probability of attaining either single or reciprocal monophyly. Unless subpopulations experience no genetic interchange, one would expect such structure to delay attainment of GS status because it will take longer for alleles to spread throughout the entire species. Wakeley (2000) provides a mathematical treatment of the relationship between population substructure and the time to coalescence when there are many demes.

The general conclusion is that if one adopts a GS criterion that requires 100% reciprocal monophyly in a sample of genes from a pair of sister taxa, it will take a long time to achieve this criterion using a moderate sample of loci unless population size is small. Because a sample is often used to assess the genealogical condition of the entire genome, one is likely to draw erroneous conclusions about GS status if one uses only a few loci. Moreover, if one requires reciprocal monophyly of every locus in the genome rather than monophyly in a small sample of loci, genealogical speciation will require very long periods of time if balancing selection occurs at any locus. Because using the ''100% monophyly'' criterion for an entire genome requires the absurd practice of combining chimpanzees with humans within one genealogical species, and rats with mice in another, it is advisable to adopt a GS criterion that requires less than 100% monophyly for either a sample of loci or the entire genome. This of course involves an arbitrary criterion for identifying a GS, but still allows one to delineate evolutionary groups with a high degree of relatedness among their members.

Using a less stringent criterion, so that only a fraction of loci in a sample (presumably greater than 50%) need be monophyletic to recognize a single GS or a pair of GSs, has the advantage that the time to partial monophyly reaches a limit as the number of loci becomes infinite (in our case, 3.76 N generations for >50% monophyly and 8.72 N generations for >95% monophyly). GS status is therefore reached in a reasonable period, and is much more likely to approximate the time to biological speciation (the attainment of reproductive isolation) than when one uses the criterion of 100% monophyly.

Our results show clearly that adding more loci to a given sample gives more accurate identification of GSs (i.e., identifications that are unlikely to change when sample sizes are increased) than does sampling more alleles while keeping the number of genes unchanged. Thus when devising genetic strategies for identifying GSs, the payoff from adding more

genes is substantially larger than the payoff from adding more alleles.

A clear lesson from our results is that one should be cautious about recognizing genealogical species using only mitochondrial or chloroplast DNA. Such DNA becomes monophyletic more rapidly than does a single nuclear gene, and far more rapidly than does a sample of several nuclear genes. Therefore, the use of mtDNA or cpDNA alone is not a good strategy for assessing reciprocal monophyly unless population divergence is very ancient.

Another reason for avoiding organelle DNA is its greater potential for becoming monophyletic by selective sweeps. Advantageous mutations occurring in mtDNA or cpDNA will cause the entire organelle genome to become monophyletic because such genomes have little or no recombination. Although selective sweeps will also occur in nuclear DNA, causing monophyly for regions linked to the selected locus, recombination will whittle away the section of genome that becomes monophyletic through linkage. It is therefore possible—although this needs theoretical study—that advantageous mutations occurring throughout the genome will lead to monophyly for mtDNA and cpDNA much sooner than for nuclear DNA, making organelle genomes even less useful for recognizing genealogical species. Possible support for this idea comes from statistical tests for selection, which show that neutrality is rejected more often for segments of mtDNA than for segments of nuclear DNA (Weinreich and Rand 2000).

Moreover, in groups such as arthropods, infectious microorganisms like the bacterium *Wolbachia* are widespread (Werren et al. 1995). Such organisms spread by cytoplasmic transmission and cause fixation of the mitochondrial genome occurring in the originally infected individual (Turelli et al. 1992). This horizontal spread of an infectious organism throughout a group is another source of discrepancy between mtDNA-based and nuclear gene-based phylogenies. In *Drosophila simulans*, for example, the genealogy of mtDNA reflects the spread of *Wolbachia* rather than the relatedness of populations as assessed by nuclear DNA (Ballard et al. 2002). Similarly, cytoplasmic male sterility in plants can be caused by genes in mitochondria, and can spread rapidly via selection, dragging both mitochondrial and chloroplast genes along with it (Olson and McCauley 2000).

Finally, when there is gene flow between diverging populations, one may encounter the opposite problem: mtDNA may be homogenized between the populations more readily than is nuclear DNA, so that mtDNA may appear paraphyletic when nuclear genes may be relatively monophyletic. In fish, mice, and crickets, for example, mtDNA flows between taxa much more readily than does nuclear DNA (e.g., Ferris et al. 1983; Smith 1992; Bernatchez et al. 1995; Taylor and McPhail 2000; Shaw 2002). The reasons for this are unclear, but may be due to the nature of mitochondrial genes, most of which are constitutively expressed and perform internal metabolic ''housekeeping'' or protein-synthetic functions (e.g., production of tRNAs and enzymes involved in respiration and electron transport). Such functions may be largely divorced from external selective pressures, making mtDNA less responsive than nuclear genes to local environmental differences.

Foreign mtDNA segments may thus be more likely to work harmoniously in a foreign genome than would segments of nuclear DNA that may have experienced divergent natural selection in new taxa. Takahata and Slatkin (1984) show that if mtDNA is neutral, only a trickle of gene flow can cause substantial introgression of this DNA between taxa, even if there is substantial hybrid unfitness based on differences in nuclear DNA. It is worth noting that most published claims of paraphyletic species involve phylogenetic analysis based solely on mtDNA (e.g., Melnick et al. 1993; Omland et al. 2000), and analysis of nuclear loci may show that the paraphyly is not pervasive throughout the genome, particularly if one adheres to the autapomorphic species concept. Our main point, however, is that phenomena such as selective sweeps and introgression can substantially accelerate or decelerate the attainment of monophyly, and that the effects of these forces may be far greater on mtDNA than on a sample of nuclear genes.

Harrison (1998) provides an incisive discussion of the relationship between the GSC and other species concepts, including the BSC. Although biological and genealogical speciation are both accelerated by divergent natural selection and geographical isolation of taxa, there is no necessary correspondence between the time at which biological and genealogical species status is attained. However, biological speciation is likely to precede genealogical speciation if GS status requires complete reciprocal monophyly at many or all loci. There are several examples in which taxa recognized as biological species are not GSs, even under relaxed criteria. For example, *D. mauritiana* and *D. simulans* share polymorphisms at many loci, and are not genealogical species under even the 50% criterion. Nevertheless, they have been genetically isolated for roughly 250,000 years, have diverged in several morphological traits, and show multiple forms of reproductive isolation (Coyne 1983, 1984; Price 1997; Kliman et al. 2000; Ballard et al. 2002).

We recognize that the fundamental purpose of the GSC differs from that of the BSC. The former takes as its ''species problem'' the demarcation and recognition of historically related entities, whereas for the BSC the ''species problem'' is the evolutionary origin of discrete clusters of organisms that exist in sympatry. These problems are not identical: as Baum (1992, p. 1) notes, ''Many objections to biological species concepts have been proposed, of which the most relevant here is that the potential for gene exchange is only loosely coupled to historical relatedness—the central consideration of systematics.'' Nevertheless, we favor the BSC over the GSC for three reasons.

First, applying the GSC will often involve the designation of taxa as metaspecies; that is, large groups of individuals will be not be recognized as belonging to any species. Unlike many doubtful cases in the BSC, such as allopatric taxa whose species status cannot be tested in sympatry, the term ''metaspecies'' describes ''an *ontological* [our emphasis] situation (organisms that are not members of any species) rather than an epistemological one (groups that cannot be assigned to recognized species due to a lack of evidence'' (Baum and Shaw 1995, p. 297). At the moment when a peripheral isolate of a large population becomes monophyletic, all members of the now paraphyletic ancestral population instantly lose their

status as members of any species. It seems odd that, without any change in its own genetic composition, a group can lose status as a species based on the genetic coalescence of a derived population. It should be noted, however, that systematists disagree on whether the term ''metaspecies'' should be used or what entities should be characterized with the term (Baum 1992; Baum and Shaw 1995).

Second (particularly if one uses the ASC or restricted versions of the GSC that involve less-than-complete monophyly of the genome), very little of biological significance happens at the moment of genealogical speciation. What significance, for example, can one impute to a single locus becoming monophyletic (the completion of autapomorphic speciation), or to a population in which the proportion of loci showing exclusivity goes from 50% to 50.1% (the completion of genealogical speciation)? In contrast, the completion of biological speciation—the moment when gene flow between sister taxa is no longer possible—corresponds to a meaningful biological event. It is the moment when taxa become evolutionarily independent units by delimiting the spread of generally advantageous alleles (Coyne 1994). The cessation of gene flow also allows genealogies to coalesce without pollution by alleles from other taxa. Thus these reproductive barriers, along with geographical barriers, provide the isolation that allows for the genetic coalescence required for autapomorphic or genealogical speciation.

Finally, in many cases genealogical (and autapomorphic) speciation will be transitory, for the coalescence of genes does not guarantee that such species will remain intact when geographically isolated populations are once again able to exchange genes. One can envision that many small, isolated populations can quickly gain genealogical species status (for example, using the >50% monophyly criterion, a population of 50 organisms will become a genealogical species in fewer than 200 generations [Fig. 4]). But range shifts or disappearance of geographic barriers will quickly eliminate these genealogical species: they will exchange genes with others and their exclusivity will vanish. In contrast, many (but not all) forms of reproductive isolation are permanent. As Futuyma (1987) has noted, morphological differentiation among populations of a species may also be transitory, disappearing when subpopulations fuse or when gene flow increases. He proposes that the association of morphological change with speciation noted by advocates of punctuated equilibrium (Gould 2001) may result from the immunity to population fusion granted by the evolution of reproductive isolation. In a similar way, the permanence of most forms of reproductive isolation guarantees the independence of genealogies among taxa.

We do not expect that such arguments will convince advocates of the GSC to embrace the BSC, for many systematists demand a species concept based on evolutionary history. And of course many criticisms have also been leveled at the BSC (see, for example, chapters in Wheeler and Meier 2000). We will not attempt to adjudicate this acrimonious debate. Here we limit ourselves to working out the mathematical consequences of some phylogenetic species concepts, and to noting some of the problems with these concepts.

LITERATURE CITED

Avise, J. C., and R. M. Ball. 1990. Principles of genealogical concordance in species concepts and biological taxonomy. Pp. 45–67 *in* D. J. Futuyma and J. Antonovics, eds. Oxford surveys in evolutionary biology. Oxford Univ. Press, Oxford, U.K.

Ayala, F. J., and A. A. Escalante. 1996. The evolution of human populations—a molecular perspective. Mol. Phyl. Evol. 5: 188–201.

Ballard, J. W. O., B. Chernoff, and A. C. James. 2002. Divergence of mitochondrial DNA is not corroborated by nuclear DNA, morphology, or behavior in *Drosophila simulans*. Evolution 56: 527–545.

Baum, D. 1992. Phylogenetic species concepts. Trends Ecol. Evol. 7:1–2.

Baum, D., and K. L. Shaw. 1995. Genealogical perspectives on the species problem. Pp. 289–303 *in* P. C. Hoch and A. C. Stephenson, eds. Experimental and molecular approaches to plant biosystematics. Missouri Botanical Garden, St. Louis, MO.

Bernatchez, L., L. Glemete, C. C. Wilson, and R. G. Danzmann. 1995. Introgression and fixation of Arctic char (*Salvelinus alpinus*) mitochondrial genome in an allopatric population of brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 52: 179–185.

Birky, C. W., and J. B. Walsh. 1988. Effects of linkage on rates of molecular evolution. Proc. Natl. Acad. Sci. USA 85:6414–6418.

Claridge, M. F., H. A. Dawah, M. R. Wilson, eds. 1997. Species: the units of biodiversity. Systematics Association Special Volume Series 54. Chapman and Hall, London.

Coyne, J. A. 1983. Genetic basis of differences in genital morphology among three sibling species of *Drosophila*. Evolution 37:1101–1118.

Coyne, J. A. 1984. Genetic basis of male sterility in hybrids between two closely related species of *Drosophila*. Proc. Natl. Acad. Sci. USA 51:4444–4447.

Coyne, J. A. 1994. Ernst Mayr and the origin of species. Evolution 48:19–30.

de Queiroz, K., and M. J. Donoghue. 1988. Phylogenetic systematics and the species problem. Cladistics 4:317–338.

de Queiroz, K., and M. J. Donoghue. 1990. Phylogenetic systematics or Nelson's version of cladistics? Cladistics 6:61–75.

Ereshefsky, M., ed. 1992. The units of evolution. MIT Press, Cambridge, MA.

Ferris, S. D., R. D. Sage, C.-M. Huang, J. T. Nielsen, U. Ritte, and A. C. Wilson. 1983. Flow of mitochondrial DNA across a species boundary. Proc. Natl. Acad. Sci. USA 80:2290–2294.

Figueroa, F., E. Günter, and J. Klein. 1988. MHC polymorphism pre-dating speciation. Nature 335:265–267.

Futuyma, D. J. 1987. On the role of species in anagenesis. Am. Nat. 130:465–473.

Gould, S. J. 2001. The interrelationship of speciation and punctuated equilibrium. Pp. 196–217 *in* J. B. C. Jackson, S. Lidgard, and F. M. McKinney, eds. Evolutionary patterns: growth, form, and tempo in the fossil record. Univ. of Chicago Press, Chicago, IL.

Harrison, R. G. 1998. Linking evolutionary pattern and process: the relevance of species concepts for the study of speciation. Pp. 19–31 *in* D. J. Howard and S. H. Berlocher, eds. Endless forms: species and speciation. Oxford Univ. Press, New York.

Hudson, R. R. 1990. Gene genealogies and the coalescent process. Oxf. Surv. Evol. Biol. 7:1–44.

Kliman, R. M., P. Andolfatto, J. A. Coyne, F. Depaulis, M. Kreitman, A. J. Berry, M. McCarter, J. Wakeley, and J. Hey. 2000. The population genetics of the origin and divergence of the *Drosophila simulans* complex species. Genetics 156:1913–1931.

Kreitman, M. 1983. Nucleotide polymorphism at the alcohol de-

hydrogenase locus of *Drosophila melanogaster*. Nature 304: 412–417.

Leaché, A. D., and T. W. Reeder. 2002. Molecular systematics of the eastern fence lizard (*Sceloporus undulatus*): a comparison of parsimony, likelihood, and Bayesian approaches. Syst. Biol. 51: 44–68.

Mayr, E. 1963. Animal species and evolution. Harvard Univ. Press, Cambridge, MA.

Melnick, D. J., G. A. Hoelzer, R. Absher, and M. U. Ashley. 1993. mtDNA diversity in Rhesus monkeys reveals overestimates of divergence time and paraphyly with neighboring species. Mol. Biol. Evol. 10:282–295.

Neigel, J. E., and J. C. Avise. 1986. Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. Pp. 515–534 *in* E. Nevo and S. Karlin, eds. Evolutionary processes and theory. Academic Press, London.

Nixon, K. C., and Q. D. Wheeler. 1990. An amplification of the phylogenetic species concept. Cladistics 6:211–223.

Ohta, T., and M. Kimura. 1968. Linkage disequilibrium between two segregating nucleotide sites under the steady flux of mutations in a finite population. Genetics 68:571–580.

Olson, M., and M. D. E. McCauley. 2000. Linkage disequilibrium and phylogenetic congruence between chloroplast and mitochondrial haplotypes in *Silene vulgaris*. Proc. R. Soc. Lond. B 267:1801–1808.

Omland, K. E., C. L. Tarr, W. I. Boarman, J. M. Marzluff, and R. C. Fleischer. 2000. Cryptic genetic variation and paraphyly in ravens. Proc. R. Soc. Lond. B 267:2475–2482.

Price, C. S. C. 1997. Conspecific sperm precedence in *Drosophila*. Nature 388:663–666.

Rubin, G. M., and many co-authors. 2000. Comparative genomics of the eukaryotes. Science 287:2204–2216.

Shaw, K. L. 1998. Species and the diversity of natural groups. Pp. 44–56 *in* D. J. Howard and S. J. Berlocher, eds. Endless forms: species and speciation. Oxford Univ. Press, Oxford, U.K.

Shaw, K. L. 2001. The genealogical view of speciation. J. Evol. Biol. 14:880–882.

Shaw, K. L. 2002. Conflict between nuclear and mitochondrial DNA phylogenies of a recent species radiation: what mitochondrial DNA reveals and conceals about modes of speciation in Hawaiian crickets. Proc. Natl. Acad. Sci. USA. *In press*.

Smith, G. R. 1992. Introgression in fishes: significance for paleontology, cladistics, and evolutionary rates. Syst. Biol. 41: 41–57.

Takahata, N., and M. Slatkin. 1984. Mitochondrial gene flow. Proc. Natl. Acad. Sci. USA 81:1764–1767.

Tajima, F. 1983. Evolutionary relationship of DNA sequences in finite populations. Genetics 105:437–460.

Tavaré, S. 1984. Line-of-descent and genealogical processes, and their applications in population genetics models. Theor. Popul. Biol. 26:119–164.

Taylor, E. B., and J. D. McPhail. 2000. Historical contingency and ecological determinism interact to prime speciation in sticklebacks, *Gasterosteus*. Proc. R. Soc. Lond. B 267:2375–2384.

Turelli, M., A. A. Hoffmann, and S. W. McKechnie. 1992. Dynamics of cytoplasmic incompatibility and mtDNA variation in natural *Drosophila simulans* populations. Genetics 132:713–723.

Uyenoyama, M. 1995. A generalized least-squares estimate for the origin of sporophytic self-incompatibility. Genetics 139: 975–992.

Wakeley, J. 2000. The effects of subdivision on the genetic divergence of populations and species. Evolution 54:1092–1101.

Weinreich, D. M., and D. M. Rand. 2000. Contrasting patterns of nonneutral evolution in proteins encoded in nuclear and mitochondrial genomes. Genetics 156:385–399.

Werren, J. H., D. Windsor, and L. R. Guo. 1995. Distribution of *Wolbachia* among neotropical arthropods. Proc. R. Soc. Lond. B 262:197–204.

Wheeler, Q. D., and R. D. Meier, eds. 2000. Species concepts and phylogenetic theory: a debate. Columbia Univ. Press, New York.

Young, J. E., and B. I. Crother. 2001. Allozyme evidence for the separation of *Rana areolata* and *Rana capita* and for the resurrection of *Rana sevosa*. Copeia 2001:382–388.