NEW TRENDS IN PLANT SYSTEMATICS

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Reconstructing patterns of reticulate evolution in angiosperms: what can we do?

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Hybridization is thought to be an important phenomenon in angiosperm evolution, and it has been suggested that a majority of all plant species may be derived from past hybridization events (e.g., Stebbins, 1959; Raven, 1976; Grant, 1981; Arnold, 1997). In addition, there is an increasing interest in the reconstruction of reticulate patterns (e.g., Linder & Rieseberg, 2004), with increased emphasis on the need to explore multiple independent markers to investigate the origin of putative hybrid species (e.g., Hamzeh & Dayanandan, 2004; Koontz & al., 2004 and other examples listed in Table 1). Several reviews have recently been published on the process of hybridization itself or on issues indirectly related to it: Hegarty & Hiscock (2005) and Zhou & al. (2005) present an overview of molecular techniques as well as criteria for distinguishing hybrid speciation; Gross & Rieseberg (2005) evaluate the ecological genetics of homoploid hybrid speciation, and Seehausen (2004) reviewed the possible role of hybridization in adaptive radiation. Mallet (2005) presented several hybrid examples in plants and animals to discuss the evolutionary significance of hybridization. Also, many studies have been published in the past several years on the incidence and role of (allo)polyploidy in evolution (e.g., in Soltis & Soltis, 1993, 2000; Ramsey & Schemske, 1998; Otto & Whitton, 2000; Crawford & Mort, 2003; Soltis & al., 2004).

In addition to species-level hybridization, other (genome-level or molecular) evolutionary processes such as recombination, gene conversion or horizontal gene transfer can confound the phylogenetic signal in the data to such an extent that it may become non-treelike, and phylogenetic methods are not appropriate for analysis. It is best to check prior to phylogenetic analysis whether this applies, and if so, then use network methods to represent it (Bryant & Moulton, 2004).

Nevertheless, the pages of botanical systematic journals are still remarkably devoid of examples of reticulate patterns, and plant species-level relationships are predominantly depicted as trees. The question can be asked whether this is because there are no suitable tools available for detection or whether the problem is merely ignored. In this paper we will explore the current practice of dealing with hybrid terminals in published phylogenetic studies, briefly describe a selection of network-producing methods currently available, as well as discuss future possibilities of reconstructing reticulate patterns in angiosperm evolution.

Many recently published studies report the occurrence of plant hybrids in several plant genera and families, both at the polyploid and homoploid level (e.g., *Spartina* [Poaceae] in Ainouche & al., 2004; *Actinidia* [Actinidiaceae] in Chat & al., 2004; *Glycine* [Fabaceae] in Doyle & al., 2004; *Phoenix* [Arecaceae] in Gonzalez-Perez & al., 2004; *Pleione* [Orchidaceae] in Gravendeel & al., 2004; *Gagea* [Liliaceae] in Peterson & al., 2004). Ellstrand & al. (1996) surveyed frequency and taxonomic distribution of spontaneous hybridization in vascular plants in five major floras. They concluded that most hybrids are concentrated in particular families such as Poaceae, Cyperaceae and Rosaceae, and several genera within these families account for most of the hybrid species encountered (Ellstrand & al., 1996). Some life-history characteristics seemed to be associated with hybridizing taxa such as perennial habit, asexual reproductive modes, and outcrossing breeding system (Ellstrand & al., 1996; Rieseberg, 1997; Wisseman & Ritz, 2005). However, it is unclear whether the observed uneven distribution is due to intrinsic (biological) differences of the lineages involved (such as breeding system or ecological preferences), or to extrinsic factors such as extreme habitat or ecological transitions (Rieseberg, 1997; Gross & Rieseberg, 2005), or distribution pattern (the extent of sympatry with other species). Also, sampling bias could be a factor with the number of reports on hybrids influenced by the systematic attention given to

Table 1. List of representative phylogenetic studies in angiosperms which include putative hybrids.

Genus	References	Species/ acc.[1]	Ploidy level of putative hybrid	Markers used	External evidence on hybrid status[2]
Achillea	Guo & al., 2004	63/82	polyploid	nrDNA ITS; cpDNA *trnL-F*	morph; AFLP
Actinidia	Chat & al., 2004	40/79	mix	mtDNA *nad*; cpDNA *matK*, *psbC-trnS*, *rbcL*, *trnL-F*	-
Amelanchier	Campbell & al., 1997	19/26	polyploid	nrDNA ITS	morph; pl
Anacamptis	Bateman & Hollingsworth, 2004	3/4	diploid?	morphology; nrDNA ITS; cpDNA *trnL-F*; RFLP	geo
Arabis	Koch & al., 2003	3/402	triploid	nrDNA ITS; chromosome counts	morph
Armeria	Aguilar & Feliner, 2003	72/131	diploid	nrDNA ITS	morph; geo; DNA
Calopogon	Goldman & al., 2004	5/56	polyploid	cpDNA restriction analysis; nrDNA ITS, AFLP; chromosome counts	-
Cardamine	Marhold & al., 2004	17/36	diploid	nrDNA ITS; AFLP	-
Cardamine	Lihova & al., 2004	22/22	tetraploid	nrDNA ITS; cpDNA *trnL-F*	pl
Ceanothus	Hardig & al., 2002	4/23	diploid	nrDNA ITS; cpDNA *matK*; allozymes; morphology	morph
Cicer	Shan & al., 2005	9/146	diploid	AFLP	morph
Dactylorhiza	Shipunov & al., 2004	9/125	mix	cpDNA *trnL-F*, *trnS-G*, nrDNA ITS; morphology	-
Delphinium	Koontz & al., 2004	30/30	diploid	nrDNA ITS; cpDNA *trnL-F*	morph; cross
Dendrochilum	Barkman & Simpson, 2002	22/22	mix	nrDNA ITS; cpDNA *accD*	morph; geo
Elymus	Mason-Gamer, 2004	33/45	hexaploid	cpDNA *rpoA*, *trnT-L*; nDNA *GBSSI*	pl
Elymus	Helfgott & Mason-Gamer, 2004	27/27	tetraploid	nDNA *pepC*	iso
Erythronium	Allen & al., 2003	24/24	tetraploid	nrDNA ITS; cpDNA *matK*	morph; iso
Fagopyrum	Nishimoto & al., 2003	15/15	tetraploid	nDNA *Flo/Lfy, AG*; cpDNA *rbcL-accD*, *trnK*, *trnC-rpoB*	-
Gagea	Peterson & al., 2004	7/32	diploid?	cpDNA *psbA-trnH* & *trnL-F*; nrDNA ITS; morphology	morph
Gossypium	Cronn & al., 2003	13/13	diploid	cpDNA *ndhF*, *matK*, *rpl16*, *trnL-F*; nrDNA ITS; nDNA *CesA1a*, *AdhC*, *CesA1b*, A1341, G1121, G1262	morph; cross
Hippophae	Sun & al., 2002	15/15	diploid	nrDNA ITS	morph; geo; DNA
Hordeum	Petersen & Seberg, 2004	28/30	tetraploid	nrDNA *DMC1, EF-G*; cpDNA *rbcL*	pl; iso
Lepidium	Mummenhoff & al., 2004	56/56	polyploid	cpDNA *trnT-L*, *trnL-F*; nrDNA ITS	morph
Mimulus	Beardsley & al., 2004	18/18	diploid?	nrDNA ITS, ETS; cpDNA *trnL-F*	morph
Miscanthus	Hodkinson & al., 2002	3/5	triploid	nrDNA ITS; AFLP; cpDNA; FISH	pl
Mitella	Okuyama & al., 2005	12/66	diploid[3]	nrDNA ITS & ETS; cpDNA *matK*, *trnL-F*	-
Nicotiana	Chase & al., 2003	66/70	polyploid	nrDNA ITS; GISH; cpDNA *matK*	pl, morph
Paeonia	Sang & al., 1995	33/45	mix	nrDNA ITS	-
Paeonia	Sang & al., 1997	32/37	tetraploid	cpDNA *matK*; nrDNA ITS	pl
Paeonia	Sang & Zhang, 1999	12/12	tetraploid	nDNA *Adh1*	pl
Pleione	Gravendeel & al., 2004	20/20	diploid	morphology; nrDNA ITS; cpDNA *trnT-L*, morph; *trnL-F*, *matK*	geo
Populus	Hamzeh & Dayanandan, 2004	21/21	?	cpDNA *trnL-F*; nrDNA ITS	RFLP
Ranunculus	Hörandl & al., 2005	c. 200/ 200	polyploid	nrDNA ITS	morph; pl; cross
Sphagnum[5]	Shaw & al., 2005	31/136	mix	nrDNA ITS; nDNA *Leafy/Flo*; cpDNA *trnL-F*; RAPD	iso
Stephanandra	Oh & Potter, 2003	9/17	diploid	nDNA *Leafy*; nrDNA ITS; cpDNA *trnL-F*, *trnD-Y-E-T*, *matK-trnK*	-
Stylosanthes	Vanderstappen & al., 2002	28/40	tetraploid	STS[4]; nrDNA ITS; cpDNA *trnL* intron	pl; morph
Tarasa	Tate & Simpson, 2003	27/27	polyploid	cpDNA *psbA-trnH*, *trnT-L*, *matK-trnK*; nrDNA ITS	-
Viburnum	Donoghue & al., 2004	42/43	diploid	cpDNA *trnK*; nrDNA ITS	geo
Viburnum	Winkworth & Donoghue, 2004	41/41	polyploid	nDNA *GBSSI*	-
Zaluzianskya	Archibald & al., 2005	23/28	?	nrDNA ITS; cpDNA *rpl16*, *trnL-F*	-

[1]Number of ingroup species (including hybrids) /accessions used.

[2]Evidence is sometimes inferred from the publications, i.e., not stated explicitly by the authors; cross = crossing experiments; DNA = "DNA evidence" (not specified); geo = biogeographic distribution; iso = isozymes; morph = morphology; pl = ploidy level.

[3]Evidence of introgression between taxa; no specific hybrid taxon is identified.

[4]STS: nuclear sequence-tagged site PCR.

[5]Bryophyta.

particular taxa (Ellstrand & al., 1996).

When hybrid species are included in phylogenetic analyses they can affect the overall tree topology. Remarkably few studies on the behaviour of hybrids in cladistic analyses have been published. The landmark studies of McDade using artificial hybrids of *Aphelandra* (Acanthaceae) are often cited to indicate that a hybrid is not expected to disrupt phylogeny reconstruction unless the hybridization event is between divergent lineages (McDade, 1990, 1992). However, this is based on a dataset consisting of morphological markers that are mostly intermediate in state for the hybrid, causing it to be placed at a basal position relative to the most derived parent. In contrast, molecular characters do not express intermediacy but can display apomorphies of both parents simultaneously (i.e., polymorphic sites or mosaic sequences) that may cause the hybrid to be placed proximate to the most derived parent. Many such apomorphic characters shared between hybrid and parents could cause long-branch attraction (McDade, 1995). Biparentally-inherited markers expressing additivity will possibly influence tree topology (loss of resolution), tree length (increase or decrease depending on treating additivity as polymorphism or uncertainty, respectively; see Kornet & Turner, 1999), or support analysis (Simmons, 2001). Many molecular phylogenetic studies use multiple markers with different modes of inheritance (i.e., nuclear and organelle). In fact, Seehausen (2004) uses the ensuing "cytonuclear discordance" as evidence for ancestral hybridization preceding evolutionary radiation.

Phylogenetic studies are sometimes conducted excluding putative hybrids in order to avoid (a priori) expected disruptive effects on the analyses (e.g., *Cardamine* [Brassicaceae], Marhold & al., 2004 and *Calopogon* [Orchidaceae], Goldman & al., 2004) or after determination of incongruence between different gene datasets (e.g., *Gaura* [Onagraceae], Hoggard & al., 2004 and *Pleione* [Orchidaceae], Gravendeel & al., 2004). In addition, several authors have analysed their data both including and excluding the putative hybrid, in order to investigate its influence on phylogenetic reconstruction. The effects of hybrid exclusion from nrDNA ITS datasets was investigated in *Achillea* (Asteraceae) by Guo & al., 2004; *Armeria* (Plumbaginaceae) by Aguilar & Feliner (2003); *Delphinium* (Ranunculaceae) by Koontz & al. (2004); *Hippophae* (Asteraceae) by Sun & al. (2002); and *Nicotiana* (Solanaceae) by Chase & al. (2003). While the *Delphinium* and *Nicotiana* studies recorded little effect of hybrid exclusion on the analysis, the *Armeria* and *Hippophae* studies found fewer most parsimonious trees with a higher consistency index and a higher resolution in the analysis upon hybrid exclusion. In *Bikinia* (Fabaceae) exclusion of a putative hybrid caused an increase in jackknife support values for both clades containing the parental species, from 68% to 93% and from less than 50% to 77%, using AFLP data (Wieringa & Guhl, in press). The authors argue that this taxon jackknifing approach could possibly be used as a standard tool to trace undetected hybrids.

The effect of hybrid exclusion in a combined analysis of nrDNA ITS and chloroplast DNA RFLPs was investigated in *Calopogon* (Goldman & al., 2004). No effect of removal of the putative hybrid (inferred from its ploidy level) on the combined analyses was found. In contrast, Hoggard & al (2004) studied two tetraploid species of *Gaura* (Onagraceae) and found a disruptive effect on tree topology of a putative hybrid with distant parents, while no effect was seen for another hybrid with "close" parents.

Cytonuclear incongruencies have confirmed several hypotheses of suspected hybrids, for example, *Anacamptis* (Orchidaceae) by Bateman & Hollingsworth (2004); *Delphinium* (Ranunculaceae) by Koontz & al. (2004); and *Dendrochilum* (Orchidaceae) by Barkman & Simpson (2002). Comparison of discordant phylogenetic trees from independent datasets has even revealed new unexpected cases of possible hybridization (e.g., *Braya* [Brassicaceae], Warwick & al., 2004; *Stephanandra* [Rosaceae], Oh & Potter, 2003; and *Viburnum* [Adoxaceae], Donoghue & al., 2004). In addition, this approach appears promising in phylogeography (see Comes & Abbott, 2001; Franzke & al., 2004; Lorenz-Lemke & al., 2005).

Of course, incongruent phylogenetic patterns within a dataset or between datasets can have causes other than the hybrid origin of one or more of the species involved. Such causes may include incomplete lineage sorting, that is, the persistence and retention of ancestral polymorphisms through multiple speciation events (e.g., Avise, 2000; Comes & Abbott, 2001; Andreasen & Baldwin, 2003; Goldman & al., 2004), homoplasy and taxonomic sampling error (Wendel & Doyle, 1998). Therefore, hybridization should not be a "standard" interpretation when incongruencies are found. Other causes should be considered carefully because the incongruent pattern alone can never be an indicator of hybrid status.

Many examples of hybrid detection involve investigation of the additivity of nucleotides at single positions (polymorphic sites) of rDNA ITS sequences (e.g., Sun & al., 2003; Gravendeel & al., 2004; Koontz & al., 2004; Marhold & al., 2004; Peterson & al., 2004; Warwick & al., 2004). An example of intraspecific ITS additivity can be found in *Clausia aprica* (Brassicaceae) where accessions of an intermediate group showed additivity, possibly indicating hybridization (Franzke & al., 2004).

Furthermore, hybrid origin and relationship to putative parents, when not extinct, can be explored in more detail using several different markers. Morphology or

patterns of geographical distribution can provide valuable additional evidence for a hybrid origin (Hughes & Harris, 1998; Bateman & Hollingsworth, 2004; Peterson & al., 2004; Shan & al., 2005). Additionally, karyological evidence, such as chromosome counts, C-values, and GISH or FISH patterns can discriminate between parental genome donors and the hybrid relationships (Hodkinson & al., 2002; Borgen & al., 2003; Chase & al., 2003; Bures & al., 2004; Harper & al. 2004; Pires & al., 2004; Tel-Zur & al., 2004). Another line of evidence for hybrid status can be found using analyses of fragment-length polymorphisms (e.g., RFLP) or the currently more often employed PCR-based markers (e.g., AFLP, ISSR, RAPD, or PCR-RFLP). For example, an additive pattern of AFLPs and the lack of unique bands confirmed the hybrid status of a species of *Mangifera* (Teo & al., 2002). Kiew & al. (2003) used AFLP data to test hybrid origin in several taxa (*Begonia*, *Mangifera*, *Nepenthes* and *Lausium*) and these data permitted the reconstruction of relations with the putative parents. Despite many examples where AFLP data are considered useful in phylogenetic studies (e.g., Kardolus & al., 1998; El-Rabey & al., 2002; Spooner & al., 2005), the application of these data (and similar single-locus markers) in infrageneric studies requires caution. One major concern involves the difficulty in assessing homology between the co-migrating fragments of more distant taxa (El-Rabey & al., 2002; but see Crawford & Mort, 2004). Therefore, the general value of the use of these markers in assessing hybrid origin remains questionable as only the successful cases tend to get published (but see Krauss & Hopper, 2001, who report that high genetic variability made it difficult to distinguish between different hybrid scenarios). More insight is needed into the "behaviour" of AFLPs, and to this end, simulation (*in silico* AFLP, see Koopman & Gort, 2004) may become increasingly important as more complete genome sequences become available (Antonov, 2002). Recent studies of hybrids in angiosperm phylogenies are listed in Table 1, with the different hybrid detection markers used.

Ideally, additional studies, such as crossing experiments, need to be conducted to support any hybrid hypothesis. Artificial crossing experiments permit investigating the possibility of crossing of the putative parents, and also comparison of the character pattern (either molecular or morphological) in progeny of controlled crosses with that of putative hybrids. For instance, experimental crosses have been used to investigate morphology and fertility in *Solanum* (Clausen & Spooner, 1998); to compare nrDNA ITS sequences between artificial and natural hybrids in *Begonia* (Chiang & al., 2001); to determine the maternal donor of F_1 hybrids in *Phlox* (Ferguson & al., 1999); and to study genomic changes in synthetic polyploids of *Brassica* (Song & al., 1995). Even more extensive studies have been performed in sunflower hybrid species, where the genomic structure of a newly formed hybrid was compared with that of ancient hybrids to study the process of diploid hybrid speciation in *Helianthus* (Rieseberg & al., 1996). In later studies, adaptive quantitative trait loci (QTL) were compared to investigate ecological divergence and adaptive genetic variation of the hybrids (Rieseberg & al., 2003; Lexer & al., 2004). While such genomic evidence can be regarded as the best and most direct evidence for documenting the hybrid nature of a species, as well as allowing assessment of the actual mechanisms involved, such data will probably never be available for most groups on a routine basis.

HYBRID DEFINITIONS

The range of possible characteristic hybrid patterns listed above (e.g., additivity of AFLP bands, polymorphic nucleotides, incongruence between gene trees, intermediate morphology, etc.), may well not apply to each hybrid plant species as not all will "behave" in the same way. Rieseberg & Ellstrand (1993) investigated chemical, morphological and molecular characters in hybrid plants and found that hybrids can display a range of characteristics at both the morphological and molecular level. This ranges from closely resembling one parent to complete intermediacy between the parentals, and in some cases to the formation of a completely new character. The authors emphasised the unpredictable nature of character expression in hybrids, hence preventing hybrid detection based on a specific "hybrid character syndrome". Many other studies corroborate these findings with different character patterns found in different hybrids (Table 1). To our knowledge, no recent review on patterns in hybrid characters has been performed, comparable to the list of Rieseberg & Ellstrand (1993).

The other reason that no general pattern in hybrids can be inferred from published studies lies in inconsistent terminology. For example, Rieseberg and Ellstrand (1993) discriminate between "first generation hybrids", "later generation hybrids" and "hybrid species". According to the authors, the latter category is the most difficult to detect, since hybrid species are more prone to display many new and extreme characters, while F_1 hybrids will probably more often show a blend of characters of the parental species. McDade (1995) reviewed character patterns in hybrids and introduced the terms "primary hybrids" ("with simple histories and little change since origination") and "derived hybrids" (with "considerable evolutionary change since origination"). She used these categories to indicate that the amount of evolutionary change will probably define whether

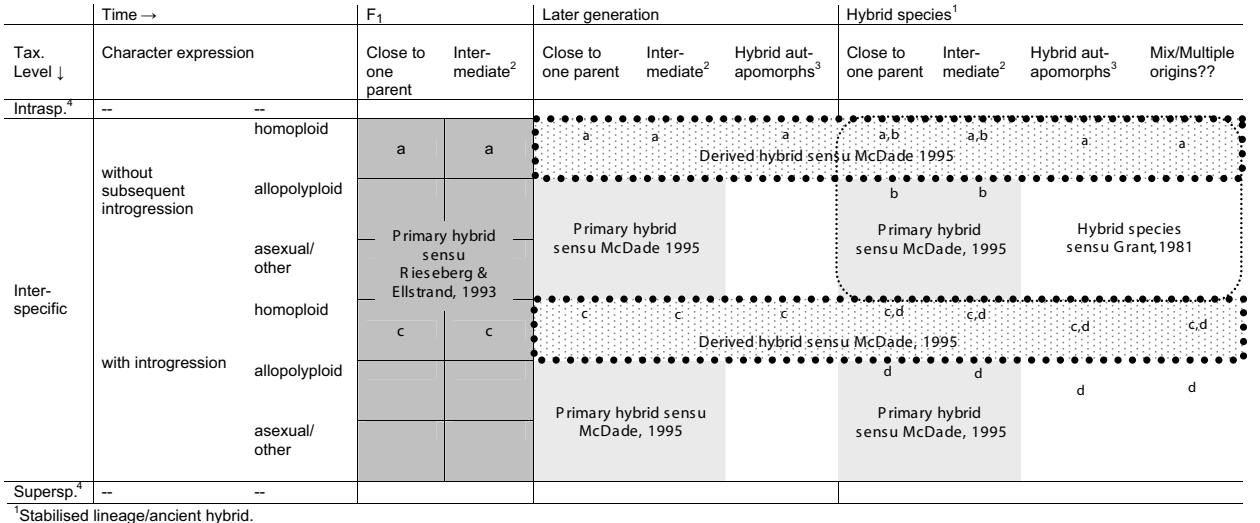
hybrids can be dealt with in systematics. "Primary hybrids" are the only category where we can expect to understand the behaviour of their characters (McDade, 1995).

Additionally, the term "hybrid" is used for a wide variety of entities (McDade, 1995), often without reference to important factors that must be considered, such as age, ploidal level and parental phylogenetic distance. Most systematic studies dealing with hybrids do not explicitly state what hybrid definition is used, but simply assume that a hybrid is a cross between different species, or define it as "interspecific" or "hybrid between species". There are, however, some studies that explicitly refer to the age of the hybrid or "stability" of the hybrid individuals. For example Bures & al. (2004) specify that they include (sterile) F_1 hybrids in their study of *Cirsium*; Koontz & al (2001) and Goldman & al. (2004) both discuss the possibility of ancient hybridization in respectively *Delphinium* and *Calopogon*, but these are exceptions.

The term "hybridization" is rarely specified, instead it is assumed that every worker knows what is meant, but it is important to note that several definitions exist. The most often used one is by Harrison (1993): "interbreeding of individuals from two populations, or groups of populations, which are distinguishable on the basis of one or more heritable characters". This definition does not require any consideration of species concepts, but most workers use the "standard" definition of a hybrid "resulting from crossing between different species". In an attempt to clarify matters, we include here a conceptual framework in which various hybrid definitions are logically arranged according to factors and scales that are of importance in hybrid formation, and hence in character evolution (Fig. 1). The two main axes here are "age of the hybrid" (whether it is a newly formed (F_1) hybrid or a more ancient and established hybrid species), and the taxonomic level of the hybrid's parents. The latter is further subdivided into relevant mechanism(s) involved during or after the process of hybridization, such as the amount of introgression and change in ploidy level. As McDade (1995) noted, the pattern of character state transmissions in hybrids and the amount of evolutionary change in characters are important for possible detection and behaviour of hybrid terminals in phylogenetic studies. Therefore, we include these factors here as well, and nest them within the different time scales.

REPRESENTING HYBRIDS IN PHYLOGENETIC ANALYSIS: VISUALISATION OF PATTERNS

As mentioned above, hybrids are sometimes excluded from phylogenetic analyses, often after incongruence testing among multiple datasets, or because the hybrid is expected to have a disruptive effect on the tree topology (e.g., Marhold & al., 2004). This approach intuitively makes sense because trees cannot depict hybrids and tree reconstruction could be confounded by their inclusion, with a polytomy a likely result (but see below). Moreover, when using packages such as Mesquite



[1]Stabilised lineage/ancient hybrid.
[2]Can be either mosaic or additive characters.
[3]'New' characters.
[4]Does not apply as intra- and superspecific hybridization falls outside standard hybrid terminology.

[a]*Helianthus* (Rieseberg & al., 1996, 2003).
[b]*Paeonia* (Sang & al., 1995).
[c]*Iris* (Arnold, 1993, 1997; Arnold & al., 1990, 1991).
[d]*Gossypium* (Wendel & al., 1991, 1995a, b; Cronn & al., 2003).

Fig. 1. How to become a hybrid? Conceptual framework of commonly-used hybrid terminology with exemplar studies indicated.

(Maddison & Maddison, 2004) or MacClade (Maddison & Maddison, 2005) for optimisation of characters, resolved trees are usually required as input. However, it would be a waste of potentially important information if the hybrid sequences were not used in such analyses. In addition, inclusion of the hybrid might not have a disruptive effect on the trees after all (see Chase & al., 2003; Guo & al., 2004; Koontz & al., 2004).

As outlined above, one solution to this problem could be to conduct analyses that both include and exclude putative hybrids and present both results (as in Sun & al., 2002; Aguilar & Feliner, 2003; Chase & al., 2003; Guo & al., 2004; Koontz & al., 2004; Wieringa & Guhl, in press). An alternative, and perhaps better approach might be to represent hybrid relationships directly in a network. This can be done by hand for relatively clear hybrid relationships (Sang & al., 1995; Hardig & al., 2002). However, for more complex situations this is not feasible and depicting reticulate evolutionary patterns (and all possible relationships) in one network would be a desirable feature in a computer package.

In analogy to the gene tree/species tree problem (Maddison, 1997), however, the question must be addressed of what is actually represented in such reticulated networks: character conflict or relationships among organisms (or species)? For instance, Bryant & Moulton (2002) characterise networks as "a representation of the data rather than a phylogenetic inference", and to "indicate whether or not the data is substantially treelike". Holland & al. (2005), on the other hand, describe their Consensus Network (see below) method as one "that generalizes the notion of consensus trees to allow conflicting evolutionary hypotheses to be displayed within a network".

While one would certainly like to distinguish between sources of phylogenetic tree incongruence, it is unlikely that the cause of the conflict can be inferred by analysing the pattern alone. Both homoplasy/sampling artifacts and hybridization (or other evolutionary processes) can give the same (sometimes incongruent) patterns. Also, hybridization need not necessarily result in a clear reticulated pattern of evolution. The data can not indicate any incongruence, for instance, when using uniparentally inherited markers or markers that show strong gene conversion. In addition, processes during or after hybridization (such as repeated secondary contact, as for instance under a post-glacial refugium expansion scenario), can make the actual split "messy", and hence the relationships more complex and difficult to resolve. Nevertheless, Seehausen (2004) argues that "adaptive radiation" (or at least "functional diversification") can be facilitated by interspecific hybridization and that such patterns can be clearly reconstructed.

Probably the best way of distinguishing between the different above-mentioned causes for the observed phylogenetic incongruences is therefore to use additional data or evidence of hybridization from other sources such as morphology, genomics and karyology. Yet, in spite of the objections outlined above, a network can be used as a starting point for investigating relationships. Whether or not hybridization is the cause, it is desirable to display the source of conflict. One way to do this is to visualise the character incongruences in a network, where a "hybrid" or "problematic terminal" can be connected to more than one other terminal or internode.

Unfortunately, although some programs exist that can deal with population-level data and possibly hybridization events (see below), no method is available that can be considered the perfect "hybrid interpreter". The only way to make progress with this problem is to seek methods to deal with complex hybrid terminals using simulation and experimental data. The common practice of leaving suspected taxa out of the analysis to avoid confounding effects on phylogenetic reconstruction will not stimulate further progress. Below we explore some of the methods currently available and infer possible solutions and suggest some future research directions.

CURRENT TOOLS FOR REPRESENTING INCONGRUENT PHYLOGENETIC PATTERNS SIMULTANEOUSLY

Posada & Crandall (2001a) listed a range of methods and software for network estimation that can possibly "take into account population-level phenomena and allow for persistent ancestral nodes, multifurcations and reticulations". Examples are the method of statistical parsimony (as implemented in the package *TCS*, Clement & al., 2000), *SplitsTree* (Huson & Bryant, 2004), and *Network* (Bandelt & al., 1995, 1999) but so far, these methods have not been used frequently in published studies of angiosperm species phylogenies. In Table 2 we list currently available and accessible methods aiming at network reconstruction. Some of these programs are character-based, such as the *Median Network* and *Median-Joining network* approach of Bandelt & al. (1995, 1999), but most other methods are distance based. Generally, programs can be distinguished by whether they are based on an algorithmic approach or use an optimality criterion. Most network methods are based on an algorithmic approach and do not explore alternative solutions. However, the *Median Network* approach displays all parsimonious solutions in one network, and it is not

Table 2. List of selected currently available network reconstruction packages.

Package	Reference	Network reconstruction method	Input data	Website
Network	Bandelt & al., 1995	Median networks[1] Median-joining networks[2]	Binary characters Multistate characters	http://www.fluxus-technology.com/
Spectronet	Huber & al., 2002	Median networks[1]	Binary characters	http://awcmee.massey.ac.nz/spectronet/index.html
Arlequin	Schneider & al., 2000	Molecular-variance parsimony[3]	Multistate characters/ haplotype frequencies	http://lgb.unige.ch/arlequin/
SplitsTree	Huson & Bryant, 2004	Split decomposition[4]	Multistate characters or distances[a]	http://www-ab.informatik.uni-tuebingen.de/software/jsplits/welcome.html
		NeighborNet[5]	Multistate characters or distances[a]	
		Consensus networks[6]	Trees[b]	
T-rex	Makarenkov, 2001	Reticulogram reconstruction[7]	Distances	http://www.labunix.uqam.ca/~makarenv/trex.html
TCS	Clement & al., 2000	Statistical parsimony[8]	Multistate characters/ haplotype frequencies	http://darwin.uvigo.es/software/tcs.html

[a]Input data are multistate characters or distances; analysis is based on distances. [b]Input data are trees; analysis is based on splits of these trees.
[1]Bandelt & al., 1995. [2]Bandelt & al., 1999. [3]Excoffier & Smouse, 1994. [4]Bandelt & Dress, 1992. [5]Bryant & Moulton, 2002, 2004. [6]Holland & Moulton, 2003. [7]Makarenkov & Legendre, 2004. [8]Templeton & al., 1992.

immediately clear whether an algorithm or criterion-based approach applies here.

Most of the programs listed in Posada and Crandall (2001a) aim at population-level data and there are several recent examples of their application. For example, multiple origins in *Glycine tomentella* were investigated by Rauscher & al. (2004) using *TCS*; representation of phylogeographic relationships in dolphins was reconstructed using *TCS*, *Network*, *Arlequin* and *SplitsTree* (Cassens & al., 2003); and patterns of genetic diversity were explored in *Scaevola plumieri* (Asteraceae) using *SplitsTree* (Barker & al., 2003). However, there are few examples of applications of using network programs at the angiosperm species-level. For instance, hybrid relationships in *Opuntia* were investigated with the *Median Network* approach of *Spectronet* (Griffith, 2003), and split decomposition (with the program *SplitsTree*) was used to study species radiation and reticulate relationships in *Ranunculus* (Lockhart & al., 2001; Hörandl & al., 2005). Also, *Median-Joining networks* (Bandelt & al., 1999) have been used for detailed analyses of introgression and hybridization zones between two species of *Populus* (Lexer & al., 2005) and *Passiflora* (Lorenz-Lemke & al., 2005).

In addition to the methods outlined above, there are several methods aimed specifically at detecting recombination (reviewed by Posada & Crandall, 2001b, 2002; Posada & al., 2002; Posada, 2002). These methods can only be used to test whether or not recombination is likely to be present in the data and do not display reticulate relationships. For instance, the new package TOPALi (see http://www.bioss.ac.uk/~iainm/topali/; Milne & al., 2004) is one of the available recombination detection programs that has several methods implemented to automatically identify recombinant sequences within DNA multiple alignments. One of these methods works by sliding a window along a sequence alignment, and measuring the discrepancy between the trees suggested by the first and second halves of the window, using distance matrix methods. If we could use these programs to "correct" phylogenetic data sets prior to phylogenetic analysis by scanning and removing recombined regions, this could prove highly useful.

Ideally, one would like to test the performance of network reconstruction methods using simulated data as has been done for several other phylogenetic methods (e.g., Suzuki & al., 2002; Douady & al., 2003; Hall, 2005). However, since many network reconstruction programs do not have a batch mode, simulation can become a cumbersome enterprise (pers. obs. and L. Nakhleh, pers. comm.). More importantly, in network simulations it is not clear what test statistic to use when comparing networks to a simulated model network. Measurements such as the partition metric (Robinson & Foulds, 1981) as used in many simulation studies (e.g., Leitner & al., 1996; Zwickl & Hillis, 2002; Piontkivska, 2004), are not available for a network. Nakhleh & al. (2003) tested the performance of *SpNet* and *SplitsTree*, using a modified version of the Robinson-Fould metric specifically designed and implemented for their experiments, but not suitable for wider use.

In another study, we will focus on the performance of selected network reconstruction methods using "real" published data (Vriesendorp & Bakker, in prep.). Here, we would like to note that despite the wealth of examples of hybrids in phylogenetic studies (Table 1), the successful use of network reconstruction seems to be restricted to the population level. This is understandable since phylogenetic DNA sequence datasets usually contain far higher levels of variation than what most methods are

designed to deal with. Moreover, the consistency and relative performance of these methods at the species level is not well understood, as outlined above.

In conclusion, the question remains whether a network representation can give a meaningful representation of species relationships, and whether such an analysis provides added value with respect to tree analysis. It remains to be seen whether network representation can be a good alternative to placement of the hybrid by hand on a phylogenetic tree (e.g., Sang & al., 1995; Whitehouse, 2002). However, when networks are not interpreted as displaying evolutionary relationships, but instead represent character conflict in the data, these packages prove very useful. For instance, uncovering data ambiguity using NeighborNet or Consensus networks in a way that (consensus) trees cannot, is a valuable addition to our phylogenetic tool palette, providing new insights in the analysis of data structure.

Many network reconstruction methods are based on a combined approach and explore incongruencies within a combined dataset (Posada & Crandall, 2001a; Linder & Rieseberg, 2004). Exploring and summarising separate datasets (or gene trees) could have preference above combining all data "a priori" and conducting simultaneous analysis. In their review, Linder & Rieseberg (2004) stress the importance of using multiple independent markers in the reconstruction of patterns of reticulate evolution in plants. The best approach appears to be to combine as many independent gene trees as possible into a species tree and infer hybrid relationships from there. A similar approach is proposed by Nakhleh & al. (in press) in their package RIATA-HGT, which enables inference of horizontal gene transfer events based on analysis of incongruence among species and gene trees.

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