

# Progenitor-derivative species pairs and plant speciation

Daniel J. Crawford

*Department of Ecology & Evolutionary Biology and Biodiversity Research Center, University of Kansas, Lawrence, Kansas, U.S.A.  
dcrawfor@ku.edu*

**Abstract** In addition to the classic allopatric model of speciation in which two lineages diverge through time, a population may “bud off” from an ancestral population and become adapted to a different habitat. The ancestral population (the progenitor or p species) remains largely unchanged in its original habitat while the population that budded off acquires novel characters and becomes the derivative (d) species. P-d species pairs are appropriate systems for studying plant speciation because they represent recent divergence, making it more feasible to identify the few differences between the taxa and to infer possible early-evolving barriers to gene flow. The evidence that has been used to designate species as p-d pairs is reviewed and evaluated. P-d species pairs are compared for several characters, with ecogeographic factors the only consistent difference between species pairs. Three genera in which p-d species pairs have been studied in some depth are used to illustrate both the advantages and challenges of studying the process of speciation. It is suggested that there are some advantages in focusing future studies on even earlier stages of divergence, in which speciation is not yet complete, and p and d populations can be identified. Efforts should focus on ever more refined genetic analyses of differences between populations, especially factors reducing effective gene flow between them.

**Keywords** progenitor-derivative species pairs; speciation

## ■ INTRODUCTION

The classic model of speciation envisions divergence of populations or races in allopatry; with little or no gene flow, the two systems will accumulate differences through time. Reproductive isolating barriers will develop in allopatry, and should the two species come into contact later, they would remain distinct, that is, pass the test of sympatry (Coyne & Orr, 2004). Assuming that sufficient time has passed since divergence, the species resulting from this process would be diagnosed as sister species in a cladistic analysis, and indeed fit the model of having diverged from a common ancestor (Gottlieb, 2003). This is the classical allopatric model of speciation and it has been widely accepted for speciation in both plants and animals (Mayr, 1942, 1970; Dobzhansky, 1970; Grant, 1981; Coyne & Orr, 2004). The primary reason for its wide acceptance is its high plausibility; because gene exchange does not occur between the diverging populations the problem of recombination countering divergence is circumvented (Grant, 1981; Coyne & Orr, 2004). Although this mode of speciation may well be common in plants, this does not preclude the possibility of other modes contributing significantly to speciation in plants. For example, polyploid and homoploid hybrid speciation are often cited as examples of sympatric speciation in plants (Coyne & Orr, 2004: chapt. 9), and while documented examples of the latter process are relatively few (Rieseberg & Willis, 2007), polyploidy is very common, with several well-known examples of recent origins of polyploid species (Otto & Whitten, 2000; Soltis & al., 2003; Abbott & Lowe, 2004). Sympatric speciation in plants not involving either hybridization or polyploidy has been the topic of recent discussion and debate (Savolainen & al. 2006a,b; Stuessy, 2006; Butlin & al., 2008; Fitzpatrick & al., 2008; Babik & al., 2009). Other modes of speciation

may likewise be more common than presently recognized, but more difficult to detect than species that originated by gradual divergence in allopatry.

Mayr (1942, 1963, 1970) proposed another model of geographical speciation in which peripheral isolates or long-range dispersal from parental populations result in the establishment of genetically isolated populations; this was termed peripatric speciation. Grant (1963: 456–459; 1981: 155–160) coined the term “quantum” speciation, which included the budding-off element of peripatric speciation, but added the elements of selection-drift from Wright (1931) and quantum evolution after Simpson (1944). According to Grant (1981: 157), “Both concepts pertained to small isolated populations, and both predicted that such populations would be favorable sites for rapid and drastic evolutionary changes.” Grant (1981: 157) contrasted the gradual conservative process of geographical speciation with the “rapid and radical” pace of quantum speciation. With this scenario, instead of two true sister species, there would be a progenitor-derivative (hereafter p-d) species pair in which the p species in the ancestral or source population would be little modified. In contrast, the d species would either exhibit a suite of characters not seen in the ancestral population or be fixed for features that are polymorphic in the p (Gottlieb 1973a, 1979, 2003).

There are two important elements in the study of p-d species pairs, and to some extent the two aspects of a successful study are in conflict. One is documenting phylogenetically that the two species are related as p-d, and the second is “catching” cases of recent speciation. With very recent divergence, populations of the two species may not be resolved phylogenetically (see below). The reasons for studying cases of recent speciation were articulated by Gottlieb (1973a) and Templeton (1982: 106), who said, “It is virtually impossible to sort out

which differences are actually associated with the process of speciation and which are consequences of evolution subsequent to the speciation process.”

The general purpose of the present paper is to review several aspects of p-d species and the study of speciation. The methods and criteria for inferring p-d species pairs will be discussed and comments provided on studies purporting to represent a p-d situation. A second focus of the paper is comparison of p-d species for several attributes such as habit, and mating system. A third topic is consideration of factors that reduce gene flow between p and d species. The final aims of this review are to provide a general critique of the contributions of p-d species to the study of plant speciation, and to discuss the direction of future studies.

### ■ DETECTING P-D SPECIES PAIRS

**Morphology.** — Morphological similarity is, in nearly all cases, the initial basis for inferring a p-d species pair. In some cases such as *Clarkia* (Lewis, 1973), *Coreopsis* (Turner, 1960; Smith, 1974), *Haplopappus* = *Xanthisma* (Jackson, 1962), and *Stephanomeria* (Gottlieb, 1973a) the pairs of species are quite similar morphologically and their initial recognition was facilitated by other data such as chromosome number differences, sterility of synthetic hybrids, and comparative morphology when grown side-by-side in cultivation. The treatments of *Coreopsis* (Strother, 2006) and *Xanthisma* = *Haplopappus* (Hartman, 2006) for the Flora of North America did not recognize *C. nuecensoides* as distinct from *C. nuecensis* and *X. ravenii* as different from *X. gracile*. In addition, Cronquist (1994) argued that the morphology of *Stephanomeria malheurensis*, the presumed d species, falls within the range of morphological variation found in the presumed p taxon, *S. exigua* subsp. *coronaria*. The author has studied the species pair in *Coreopsis* in the field and in cultivation (Crawford & Smith, 1982; Cosner & Crawford, 1990), and has seen the two *Stephanomeria* species growing side-by-side in cultivation. There was never a problem in distinguishing the two species of *Coreopsis* and, while the two species of *Stephanomeria* are similar, they are clearly discernable, with the differences articulated by Gottlieb (1973a). These are examples of p-d species pairs in which morphological similarity was used to make taxonomic judgments without apparent consideration for other available biological data (see below).

*Layia* is a notable exception to the common p-d situation of species similarity because crossing studies by Clausen & al. (1947) provided the first suggestion that populations differing in several conspicuous features of the capitula were not highly divergent genetically. Subsequent genetic studies of the morphological differences between *Layia glandulosa* and *L. discoidea* (Ford & Gottlieb, 1989, 1990), high identity at allozyme loci (Gottlieb & al., 1985), and a molecular phylogenetic analysis (Baldwin, 2005) further documented a close relationship between the two species. Regardless of the situation, i.e., either little or no morphological divergence or extensive divergence between the species, studies beyond superficial morphological comparisons are necessary to identify potential p-d pairs.

**Cytogenetics.** — The early studies extending beyond comparative morphology included cytogenetics. In most cases, hybrids were made between species, or different populations initially thought to belong to the same species, and the hybrids were found to have lowered pollen fertility (e.g., Togby, 1943; Lewis & Roberts, 1956; Vasek, 1960; Jackson, 1962; Kyhos, 1965; Small, 1971a,b; Smith, 1974). The occurrence of unusual or novel chromosome rearrangements, as inferred from meiotic pairing in synthetic hybrids, was used as evidence for the derived condition in some taxa (e.g., Lewis & Roberts, 1956; Jackson, 1962; Smith, 1974). In some instances (e.g., Lewis & Roberts, 1956; Small, 1971a,b; Smith, 1974), different chromosome numbers were observed because the chromosome rearrangements causing hybrid sterility also resulted in change in number.

**Molecular markers.** — Enzyme electrophoresis (e.g., Gottlieb, 1973a,b, 1974a; Gottlieb & Pilz, 1976; Gottlieb & al., 1985; Crawford & Smith, 1982; Crawford & al., 1985, 1993; Loveless & Hamrick, 1988; Cosner & Crawford, 1990; Wendel & Percival, 1990; Linhart & Premoli, 1993; Purdy & al., 1994; Purdy & Bayer, 1995; Edwards & Sharitz, 2000) followed cytogenetics to identify and study p-d species pairs. The two seminal allozyme papers by Gottlieb (1973a, 1974a) stimulated many subsequent studies of putative p-d species pairs because they demonstrated the relative simplicity of comparing closely related species for determining whether they showed the expected results of low divergence at allozyme loci and/or lower diversity in the d species (Gottlieb, 1973a, 1974a; Crawford & al., 1985). While it was accepted that allozymes had certain limitations that could underestimate divergence between species, at that time they were virtually the only way to assess allelic differences at individual loci (Gottlieb, 2003).

More recently, PCR-based markers have been used in a few studies (Perron & al., 1995; Purps & Kadereit, 1998; Pfosser & al., 2002; Kadereit & Kadereit, 2005) to examine p-d species pairs. Perron & al. (2000) used diversity in sequence-tagged-site (STS) loci to compare p-d species of *Picea*. Jaramillo-Correa & Bousquet (2003) employed mitochondrial DNA fragments to assess divergence between and diversity within *Picea mariana* and *P. rubens*.

**Molecular phylogenetics.** — Phylogenetic relationships are now routinely hypothesized from molecular data, especially DNA sequences (e.g., Soltis & Soltis, 1998; Andreasen & Baldwin, 2003; Ford & Gottlieb, 2003; Samuel & al., 2003). However, as discussed below, it is often difficult to obtain strong confirmation of p-d relationships from molecular-phylogenetic studies (Rieseberg & Brouillet, 1994; Baldwin, 2005). Because phylogenetic analyses resolve branching patterns, they usually do not provide compelling evidence for documenting a p-d relationship, especially the direction of evolution. The strongest phylogenetic evidence comes from populations of one of the species (d) being nested within populations of another species (p). This, of course, makes the p species paraphyletic, but with time lineage sorting and extinction will result in monophyly (Rieseberg & Brouillet, 1994). Another phylogenetic pattern that is concordant with **but not proof of** a p-d situation is when populations of the two taxa are resolved as sister species in a

strongly supported clade. Lastly, populations of the two species may occur in a robustly supported clade but with no internal resolution, and this likewise is concordant with but not strong evidence for a p-d pair. As discussed below, all of these situations have been found in presumed p-d species pairs. While molecular phylogenetic data may not provide compelling data for determining evolutionary direction of p-d relationships, they are important in documenting that the two taxa are closely related (as outlined above) before other critical comparisons are made. In other words, there must be compelling data that the species being compared are more closely related to each other than they are to other species; if this were not the case, it would obviously invalidate any further comparative studies under the assumptions of p-d relationships.

**Determining direction of evolution.** — In the absence of rigorous molecular-phylogenetic data for inferring the p and d species in any pair, other data must be used. As discussed above, enzyme electrophoresis has proven useful, with the d species having a subset of the allelic variation detected in the p species (Gottlieb, 1973a, 1974a, 1984; Crawford, 1985). Another feature that may sometimes be helpful is breeding system, in which there has been breakdown of self-incompatibility (SI, see below); at lower taxonomic levels, there is essentially no question that loss rather than gain of SI is the direction of evolutionary change (Barrett & al., 1997; Goodwillie, 1999; Beck & al., 2006; Igic & al., 2008). Species occurring locally on “unusual” substrates, such as serpentine or soils contaminated with heavy metals, or found in small areas at the edges of a widely distributed species are most likely derived (Lewis, 1962, 1966, 1973; Gottlieb, 1973a, 2003; Macnair & Gardner, 1998; Baldwin, 2005). The “unusual” substrates, especially soils contaminated with heavy metals, are often of more recent origin than the substrate on which the presumed p species occur.

## ■ EVALUATION OF PROPOSED P-D SPECIES PAIRS

Gottlieb (2003: table 1) provided a list of 31 proposed examples of p-d species pairs, and discussed three of them, all ones he and collaborators have studied in detail. In addition, references to the evidence used to infer p-d relationships were given. To the proposed examples of p-d pairs listed by Gottlieb (2003: table 1), several additional pairs have been added and are shown in bold, and those species which are now viewed as doubtful p-d situations are indicated by an asterisk (Table 1). In addition, there have been a number of recent publications relevant to relationships between the species pairs presented in Gottlieb (2003: table 1). Thus, it seems useful to summarize molecular phylogenetic data bearing on relationships between suggested p-d species pairs. Literature citations are given in the following discussion and in Table 1. Several caveats are in order before discussing individual cases. In many studies, only one population per species was included in analyses, and those species are designated by an asterisk in the following paragraph. Second, the sequences used vary among studies, with some using employing regions from the plastid, some

using only nuclear (usually ITS) sequences, and some both. In all cases, the emphasis in the following discussion is on whether there are strong data **against** a close relationship between species. The reader is referred to the original publications for more detailed information.

The only molecular phylogenetic study providing strong support for a p-d relationship is Baldwin (2005) for *Layia glandulosa*-*L. discoidea* (Table 1), in which populations of the d species are nested within those of the p species. There are several genera in which the proposed p-d species are resolved as sister taxa: *Clarkia lingulata*\*-*C. biloba*\* (Sytsma & Gottlieb, 1986; Ford & Gottlieb, 2003); *Crepis neglecta*\*-*C. fuliginosa*\* (Enke & Gemeinholzer, 2008); *Gossypium davidsonii*\*-*G. klotzchianum*\* (Seelanan & al., 1997); *Lemna valdiviana*\*-*L. yungensis*\* (Les & al., 2002); *Rhaphithamnus spinosa*\*-*R. venustus*\* (R.G. Olmstead, pers. comm.); *Senecio nebrodensis*\*-*S. viscosus*\* (Pelser & al., 2007); *Wolffiella hyalina*\*-*W. repanda*\* (Les & al., 2002). In several genera where more than one population per species was examined, all populations of both species occurred in one clade but not all populations of each species were resolved as clades; these include: *Coreopsis nuecensoides*-*C. nuecensis* (Mason-Gamer & al., 1991); *Lasthenia minor*-*L. maritima* (Chan & al., 2001); *Oenanthe aquatica*-*O. coninoides* (Kadereit & Kadereit, 2005; Jensch & Poschlod, 2008); and *Stephanomeria exigua* subsp. *coronaria*-*S. malheurenensis* (Lee & al., 2002).

Only one population of each species of *Picea* was sampled, and they were together in the same clade that included a third species of *Picea* (Ran & al., 2006). Each pair of *Acer* species (one population each) occur in a clade, but in both cases other species are in the two clades, with little or no resolution within either clade (Pfosser & al., 2002). In these two genera, the data do not rule out a p-d relationship between species, but at the same time do not provide support for such a relationship. The results of a molecular phylogenetic study by Hoddard & al. (2004) suggest that *Gaura longiflora* and *G. demareei* are not sister species; the two taxa are in different clades in the combined nuclear-plastid tree, with *G. demareei* in a strongly supported clade with two other species. Relationships between the two species of *Aletes* (*A. acaulis*-*A. humilis*) are not well resolved; in some analyses, the two occur in a large, highly unresolved clade, together with several other species of *Aletes* as well as other genera (Downie & al., 2002). In other trees the proposed p-d species are in different clades with other genera, but there is little support for the clades. Available data suggest that the two species are not related as p-d. (Downie & al., 2002).

Of all the proposed p-d species pairs for which relevant molecular phylogenetic studies are available, there is strong evidence against a p-d relationship for the two species of *Gaura* and there is considerable doubt about the two species of *Aletes*. In the genera *Acer* and *Picea*, the phylogenetic analyses are inconclusive on whether the species are related as p-d. While molecular phylogenetic data are apparently lacking for the *Clarkia rubicunda*-*C. franciscana* species pair, enzyme electrophoresis (Gottlieb, 1973b) and a gene duplication in the proposed p but not the d species (Gottlieb, 1974b) argue against a p-d relationship (Table 1).

**Table 1.** Potential progenitor/derivative species pairs with references (modified from Gottlieb, 2003, with additional species pairs in bold). Asterisks indicate doubtful p-d species pairs (see text).

| Family              | Progenitor/derivative species with references   |
|---------------------|---|
| Aceraceae           | <i>Acer mono</i> / <i>A. okamotoanum</i> (Pfosser & al., 2002)<br><i>A. pseudosieboldianum</i> / <i>A. takesimense</i> (Pfosser & al., 2002)  |
| Agavaceae           | <i>Camassia scilloides</i> / <i>C. angusta</i> (Ranker & Schnabel, 1986)  |
| Apicaceae           | <i>Aletes acaulis</i> / <i>A. humilis</i> * (Linhart & Premoli, 1993; Downie & al., 2002)<br><i>Oenanthe aquatica</i> / <i>O. conioides</i> (Kadereit & Kadereit, 2005; Jensch & Poschlod, 2008)  |
| Araceae (Lemnaceae) | <b><i>Lemna valdiviana</i></b> / <b><i>L. yungensis</i></b> (Landolt, 1998; Les & al., 2002; Crawford & al., 2006)<br><b><i>Wolffiella hyalina</i></b> / <b><i>W. repanda</i></b> (Landolt, 1994; Les & al., 2002; Crawford & al., 2006)  |
| Alismataceae        | <i>Sagittaria isoetiformis</i> / <i>S. teres</i> (Edwards & Sharitz, 2000)  |
| Asteraceae          | <i>Chaenactis glabriscula</i> / <i>C. fremontii</i> & <i>S. stevioides</i> (Kyhos, 1965)<br><i>Cirsium canescens</i> / <i>C. piticheri</i> (Loveless & Hamrick, 1988)<br><i>Coreopsis nuecensoides</i> / <i>C. nuecensis</i> (Turner, 1960; Smith, 1974; Crawford & Smith, 1982; Cosner & Crawford, 1990; Mason-Gamer & al., 1991)<br><i>Crepis neglecta</i> / <i>C. fuliginosa</i> (Tobgy, 1943; Enke & Gemeinholzer, 2008)<br><i>Haplopappus ravenii</i> / <i>R. gracilis</i> (= <i>Xanthisma gracile</i> ) (Jackson, 1962)<br><i>Lasthenia minor</i> / <i>L. maritima</i> (Ornduff, 1966, 1976; Crawford & al., 1985; Vasey, 1985; Chan & al., 2001)<br><i>Layia glandulosa</i> / <i>L. discoidea</i> (Clausen & al., 1947; Clausen, 1951; Gottlieb & al., 1985; Ford & Gottlieb, 1989, 1990; Baldwin, 2005)<br><i>Senecio nebrodensis</i> / <i>S. viscosus</i> (Kadereit & al., 1995; Purps & Kadereit, 1998; Pelsner & al., 2007)<br><i>Stephanomeria exigua</i> subsp. <i>coronaria</i> / <i>S. malheurensis</i> (Gottlieb, 1973a; Lee & al., 2002) |
| Caryophyllaceae     | <i>Stellaria longipes</i> / <i>S. arenicola</i> (Purdy & al., 1994)   |
| Liliaceae           | <i>Erythronium albidum</i> / <i>E. propullens</i> (Pleasants & Wendel, 1989)  |
| Malvaceae           | <i>Gossypium davidsonii</i> / <i>G. klotzchianum</i> (Wendel & Percival, 1990; Seelanan & al., 1997)  |
| Onagraceae          | <i>Clarkia biloba</i> / <i>C. lingulata</i> (Lewis & Roberts, 1956; Lewis, 1961, 1962; Gottlieb, 1974a; Sytsma & Gottlieb, 1986; Ford & Gottlieb, 2003)<br><i>C. borealis</i> / <i>C. mosquinii</i> (Small, 1971a,b)<br><i>C. mildrediae</i> / <i>C. stellata</i> (Mosquin, 1962)<br><i>C. mosquinii</i> / <i>C. australis</i> & <i>C. virgata</i> (Small, 1971a,b; Gottlieb & Ford, 1999)<br><i>C. rubicunda</i> / <i>C. franciscana</i> * (Lewis & Raven, 1958; Gottlieb, 1973b, 1974b)<br><i>C. unguiculata</i> / <i>C. exilis</i> , <i>C. springvillensis</i> & <i>C. tembloriensis</i> (Vasek, 1958, 1960, 1964, 1968; Holsinger, 1985)<br><i>Gaura longifolia</i> / <i>G. demareei</i> * (Gottlieb & Pilz, 1976; Carr & al., 1986; Hoddard & al., 2004)   |
| Phrymaceae          | <i>Mimulus guttatus</i> / <i>M. cupriphilus</i> , <i>M. nudatis</i> & <i>M. pardalis</i> (Macnair, 1989; Macnair & Cumbes, 1989; Macnair & Gardner, 1998)   |
| Pinaceae            | <i>Picea mariana</i> / <i>P. rubens</i> (Perron & al., 2000; Jaramillo & Bousquet, 2003; Ran & al., 2006)   |
| Salicaceae          | <i>Salix alaxensis</i> / <i>S. silicicola</i> (Purdy & Bayer, 1995)   |
| Verbenaceae         | <b><i>Rhaphithamnus spinosa</i></b> / <b><i>R. venustus</i></b> (Crawford & al., 1993; Sun & al., 1996; R.G. Olmstead, pers. comm..)  |

## ■ COMPARISONS OF SPECIES PAIRS

Assuming that all the species pairs in Table 1 are related as p-d, except the species of *Aletes* and *Gaura*, and *Clarkia rubicunda*-*C. franciscana* comparisons of similarities-differences between p and d species for the different pairs can be made.

**Habit.** — The habit of the different p-d pairs includes woody (*Acer*, *Gossypium*, *Picea*, *Rhaphithamnus*, *Salix*), and herbaceous perennials (*Camassia*, *Cirsium*, *Erythronium*, *Lemna*, *Sagittaria*, *Stellaria*, *Wolffiella*) and annuals (*Chaenactis*, *Clarkia*, *Crepis*, *Haplopappus*, *Lasthenia*, *Layia*, *Stephanomeria*). There are more examples of annuals than herbaceous perennials, but this is due in large measure to the several examples from the genus *Clarkia* (Table 1). Otherwise, all habits are well represented in p-d studies.

In most cases, the p and d species have the same habit, but there are exceptions. *Senecio nebrodensis* is a perennial, while its presumed d species, *S. viscosus*, is an annual (Kadereit & al., 1995). In the presumed p-d species pair *Coreopsis nuecensoides*-*C. nuecensis*, the d is an obligate annual in both nature and cultivation whereas the p species (*C. nuecensoides*) appears to be only an annual in nature but behaves as a weak perennial in cultivation, where it forms rosettes and perennating buds for several years and develops a very thick rootstock (Smith, 1974; Crawford & Smith, 1982; D.J. Crawford, unpub. data). In several cases from the genus *Mimulus*, the d species are obligate annuals whereas the p species (*M. guttatus* in all cases) varies from perennial to facultative annual to obligate annual, depending on the situation in nature (Macnair, 1989; Macnair & Gardner, 1998).

**Mating systems.** — Mating systems are known or can be inferred for some of the species pairs, and in over half of them the d species appear capable of some form of self-fertilization, varying from mixed mating to selfing. In three instances, the p species are SI and the d species are self-compatible (SC) and highly selfing: *Lasthenia* (Ornduff, 1966, 1976; Vasey, 1985); *Senecio* (Kadereit & al., 1995; Purps & Kadereit, 1998); and *Stephanomeria* (Gottlieb, 1973a). Two d species of *Mimulus* are SC like their presumed p species, but they can self-pollinate while a third d species requires a pollinator (Macnair, 1989; Macnair & Cumbes, 1989; Macnair & Gardner, 1998). There are several instances where both the p and d species are SI and thus highly if not totally outcrossing; these include *Coreopsis* (Smith, 1974), *Crepis* (Tobgy, 1943), *Haplopappus* (Jackson, 1962), and *Layia* (Clausen & al., 1947). Loss of SI and the ability to self could promote speciation in at least two ways. Selfing would facilitate rapid fixation of differences in the d species, and it would reduce gene flow between the p and d species (Gottlieb, 1973a; Grant, 1981; Levin, 2000). While the ability to self is a more common condition than obligate outcrossing in d species, there are nevertheless well-documented examples of SI d species.

## ■ POTENTIAL BARRIERS TO GENE FLOW

**Ecogeographic factors.** — A nearly universal contrast between p and d species is that the latter are more restricted in distribution and/or occur on soils with “unusual” features.

Species of *Mimulus* (Gardner & Macnair, 1998) and *Layia* (Clausen & al., 1947) grow on serpentine soils, and two species of *Mimulus* inhabit soil contaminated by heavy metals (Macnair, 1989; Macnair & Cumbes, 1989; Macnair & Gardner, 1998). The d species *Lasthenia maritima* flourishes on soils heavily modified by seabirds where very few other species are found (Ornduff, 1965, 1966, 1976; Vasey, 1985). In all these cases, and especially in instances of heavy metal contamination from mine tailings, the habitats of the d species are more recent than those of the p species. In some cases such as *Erythronium* (Pleasants & Wendel, 1989), *Sagittaria* (Edwards & Sharitz, 2000), *Salix* (Purdy & Bayer, 1995), and *Stellaria* (Purdy & al., 1994) the d species occur in habitats available subsequent to glaciation. In *Oenanthe*, the d species, *O. conioides*, is of restricted occurrence and grows in estuaries of a river with daily tidal fluctuations while the p species (*O. aquaticus*) is more widely distributed in water with unpredictable, rare tidal fluctuations (Kadereit & Kadereit, 2005; Jensch & Poschold, 2008). Seed germination increased in each species under conditions simulating the situation in nature of fluctuating and non-fluctuating water. The d species of *Acer* (Pfosser & al., 2002), *Gossypium* (Wendel & Percival, 1990), and *Rhaphithamnus* (Crawford & al., 1993; Sun & al., 1996) are island endemics, and thus the result of dispersal from continental source areas.

Landolt (1987) reviewed ecogeographical differentiation in the aquatic plant family Lemnaceae (or subfamily Lemnoideae of Araceae). Subsequent molecular phylogenetic studies have identified sister species that are not highly differentiated (Les & al., 2002). In two pairs of duckweed species (*Lemna valdiviana*-*L. yungensis* and *Wolffiella hyalina*-*W. repanda*), there are ecogeographic differences between the species. The d species of the former pair occurs in an unusual habitat (nutrient poor water) within the distribution range of the p species (Landolt, 1998). In the latter pair, the d species, *Wolffiella repanda*, is of restricted occurrence on the edge of the range of the p species in southern Africa where it occurs in smaller seasonally dry waters (Landolt, 1994; Les & al., 2002; Crawford & al., 2006).

Given the ecogeographic factors distinguishing most of the p-d species pairs, it appears likely that they are or have been important in reducing gene flow. One of the notable exceptions is *Stephanomeria*, where, despite intensive studies, no spatial segregation of the p and d species has been documented (Gottlieb, 2003).

**Other potential prezygotic barriers to gene flow.** — In addition to ecogeographic factors and breeding systems (selfing, discussed above), there are several other prezygotic factors that may serve to reduce gene flow between p-d species. The two species of *Camassia* differ in flowering time (Ranker & Schnabel, 1986), and in one p-d species pair of *Mimulus* the flowers are of different sizes and have different pollinators (Macnair & Gardner, 1998). With these notable exceptions, data on potential prezygotic barriers to gene flow (other than ecogeographic) are lacking in studies of p-d species pairs.

**Postzygotic barriers to gene flow.** — Postzygotic barriers to gene flow between p-d species pairs have been assessed by producing (or attempting to produce) synthetic interspecific hybrids. In genera such as *Chaenactis* (Khyos, 1965), *Clarkia*

(e.g., Lewis & Roberts, 1956; Mosquin, 1962; Vasek, 1964, 1968; Small, 1971a,b), *Coreopsis* (Smith, 1974), and *Haplopappus* (Jackson, 1962) very low pollen fertility in F<sub>1</sub> hybrids caused by different chromosome structure in the species pairs has been elegantly documented. Not surprisingly, postzygotic barriers to gene flow between p and d species have been most extensively documented for annuals, and are largely non-existent for woody perennials; a notable exception is *Gossypium davidsonii*-*D. klotzschianum* in which the two species were shown to be highly interfertile, but as indicated earlier, one of the species is an insular endemic and thus they are spatially separated (Wendel & Percival, 1990).

## ■ PAST AND FUTURE P-D STUDIES

Any evaluation of prior studies of putative p-d species pairs should focus on whether the most closely related species were compared, and whether there is evidence for a p-d relationship, including the direction of evolution. If these criteria are met, then a valid evaluation of what insights these studies offered into divergence and speciation can be made. The review presented above indicates that in most cases suggested p-d species pairs are more closely related to each other than to any other species. With one or two exceptions, molecular phylogenetic studies are congruent with (but do not provide compelling data for) p-d relationships. In most cases, data in addition to molecular phylogenetics are useful for inferring a p-d relationship.

Given that most proposed p-d species pairs are correct (or at least not shown to be incorrect), the major limitation of nearly all past studies has been the lack of data beyond morphological and some molecular (usually allozymes) comparisons. For some of the organisms, such as woody plants or reduced aquatics, studies beyond what has been done would be difficult. The comparisons of different p-d species pairs provide few generalizations about differences between the pairs; the notable exception is ecogeographic factors. There are, however, three genera in which studies of greater depth have been executed; they include *Clarkia biloba*-*C. lingulata*, *Layia glandulosa*-*L. discoidea*, and *Stephanomeria exigua* subsp. *coronaria*-*S. malheurenensis*, all of which were discussed by Gottlieb (2003). These genera provide insights into the advantages as well as the challenges of understanding speciation, even when using what appear to be recently diverged p-d taxa.

*Stephanomeria exigua* subsp. *coronaria* and *S. malheurenensis* are very similar morphologically, and evidence from molecular markers (Gottlieb, 1973a; Lee & al., 2002) suggests very recent divergence. Despite the many similarities between the taxa, Gottlieb and collaborators (reviewed in Gottlieb, 2003) found a number of different factors, both pre- and postzygotic, that reduce gene flow between the two species (Gottlieb, 1973a). This makes it a challenge to identify the order in which the different factors appeared in the evolution of the d species. Gottlieb (1973a, 2003) is of the opinion that the breakdown of SI was likely the first event because a SC plant of the p (*S. exigua* subsp. *coronaria*) species was found at the same locality where the d species *S. malheurenensis* occurs, indicating

that the requisite mutation was in the population. Selfing following the breakdown of the SI system would allow the rapid evolution of other characters distinguishing the two species because it would reduce gene exchange between the two. The order of appearance of the other characters distinguishing the two taxa and the relative contributions of other factors, such as cross-incompatibility and chromosome restructuring, to reproductive isolation are not known (Gottlieb, 2003).

The totality of morphological (Lewis & Roberts, 1956) and molecular (Gottlieb, 1974a; Sytsma & Gottlieb, 1986; Ford & Gottlieb, 2003) evidence documents high genetic similarity between the p-d species pair *Clarkia biloba*-*C. lingulata*. The only isolating factors that have been elucidated are the extensive chromosome rearrangements that render F<sub>1</sub> hybrids between species highly sterile (Lewis & Roberts, 1956). The two known populations of the d species *C. lingulata* grow in close proximity to populations of the much more widespread d species *C. biloba*. Prolonged efforts to elucidate ecogeographical or phenological differences between the species failed to identify possible isolating factors that may have acted or continue to act to maintain the integrity of the two species (Lewis, 1961, 1966, 1973; Lewis & Raven, 1958).

The *Layia glandulosa*-*L. discoidea* situation is similar but differs in several important respects from the other two genera. Molecular data for *Layia glandulosa*-*L. discoidea*, like examples from *Clarkia* and *Stephanomeria*, indicate little divergence between the species (Ford & Gottlieb, 1989; Baldwin, 2005), but *Layia* differs from the other two genera in features that make it more amenable to additional studies of species divergence. One is its occurrence on a different substrate, providing a likely ecogeographic isolating factor. Despite considerable morphological divergence the species have genomes that appear to recombine freely (Clausen & al., 1947; Clausen, 1951; Gottlieb & Ford, 1987; Ford & Gottlieb, 1989, 1990; Gottlieb, 2003), thus facilitating genetic analysis of character differences (Gottlieb & Ford, 1987; Ford & Gottlieb, 1989, 1990).

As summarized by Baldwin (2005), data from several sources not only indicate that *L. glandulosa* is the p of *L. discoidea* but identify variants within the former species as displaying the likely ancestral characters for the latter species. A molecular phylogenetic study provides strong evidence that *L. discoidea* (which lacks ray florets) is most closely allied to yellow-rayed forms of *Layia glandulosa* that occur nearby in the southern coast ranges of California. These data are concordant with crossing studies by Clausen & al. (1947), Gottlieb & al. (1985) and Ford & Gottlieb (1990) indicating that there is a gene in the rayless *L. discoidea* that produces yellow-rayed hybrids when crossed with white-rayed *L. glandulosa*. Lastly, there are collections of *L. glandulosa* that approach *L. discoidea* morphologically (smaller stature, very reduced or unusual ray florets), and occur on soil with some serpentine influence (Baldwin, 2005). All of these observations indicate that features incorporated into *L. discoidea* are present in populations of *L. glandulosa* in the immediate area. *Layia* exhibits two obvious candidates for reducing gene flow between the species: edaphic preference and possibly different pollinators for the two capitula types (rays and rayless) (Baldwin, 2005). However,

assuming that one or both of these features are functioning in reducing gene flow, the order in which they evolved (or indeed whether they evolved in concert), and the relative role of each in limiting gene flow remain to be determined.

Progress in studying speciation, as with other research in plant evolution, results from novel ways of thinking about or studying the process, and new tools for investigating it. Obviously, shifts in thinking and technological advances may, and probably often do, go hand-in-hand. Future studies of p-d species pairs should build on past contributions while at the same time considering new perspectives.

In a recent wide-ranging review of speciation, Sobel & al. (2010) presented several areas of research that would enhance an understanding of the process. One of their points is that better estimates of the strength of ecogeographic isolating factors are needed. A second suggestion is to compare recently diverged species for common barriers to gene flow in order to infer which barriers evolved first. Determining what traits are responsible for reduced gene flow, and elucidating adaptive traits that, either directly or indirectly, promote reproductive isolation are suggested as foci for future research (Sobel & al., 2010). Another important goal is to determine the genetic basis (number and effect of loci or genomic regions) of barriers to gene flow. A last topic mentioned by Sobel & al. (2010), is the need for more information on the role of chromosomal rearrangements in speciation. Aspects of some of these points will be included in the following discussion.

As emphasized in the present review, it has long been recognized (e.g., Gottlieb, 1973a; Templeton, 1982) and has recently been re-emphasized (Sobel & al., 2010) that successful studies of speciation must focus on cases of recent divergence, so that early evolving barriers important in divergence can be identified. This theme has been carried further with the suggestion that understanding the initial factor(s) in divergence entails examining cases where speciation is not yet complete. Via (2009: 9939) opines, “By analyzing partially reproductively isolated ecotypes or races, the genetic changes contributing to reproductive isolation can be studied before they become confounded by additional genetic differences between species that accumulate after speciation is complete.” Via (2009) referred to this as the “magnifying glass” approach because it examines in detail isolating factors between populations that represent partially isolated and divergent ecotypes. Via (2009) contrasts the “magnifying glass” with the “spyglass” approach in which one looks back into time from the perspective of the present in which speciation has been completed (or is nearly complete) and inferences are made about how the process occurred.

The question of when speciation is complete may not always be easy to answer. It is generally considered that primary divergent speciation involves stages along a continuum and several guidelines have been suggested to infer stages (Nosil, 2008; Nosil & al., 2009). The stages in the process of speciation include level of reproductive isolation (ranging from absent to complete), the degree of genotypic clustering (from unimodal to strongly bimodal), weak to strong lineage sorting, and the steepness of geographical or ecological clines (Nosil, 2008; Nosil & al., 2009). There are several potentially problematic

issues with attempting to determine stage of speciation, particularly with plants. First, some regions of the genome and thus some characters/markers move readily between even “good” species, yet they do not compromise the integrity of species boundaries (Rieseberg & al., 1999; Minder & al., 2007; Yatabe & al., 2007; Kane & al., 2009). The uncritical use of some largely neutral markers without ecological and morphological observations could be misleading with regard to the stage of speciation (Kane & al., 2009). Also, regardless of the eventual contribution of the initially evolving isolating barrier to total reproductive isolation once speciation is complete, knowledge of the initial isolating factor (or factors) is critical to understanding both the temporal order of the origin of barriers and its (their) contribution to total isolation. Prezygotic barriers such as ecogeographic factors or pollinators are often viewed as very important in reproductive isolation in plants (Ramsey & al., 2003; Rieseberg & Willis, 2007; Sobel & al., 2010). Since prezygotic factors act early in life history, their relative contributions toward total reproductive isolation would likely be greater than later acting components of isolation simply because factors acting later in the life-cycle can only reduce gene flow that is not stopped by earlier barriers (Ramsey & al., 2003; Sobel & al., 2010).

The best cases in plants for studying the initial stages of p-d speciation with the “magnifying glass” approach may come from populations or population systems (ecotypes) in which a factor or a very small number of factors potentially reducing gene flow can be identified. Some of the best-known examples (there may be many excellent examples yet to be discovered) come from plants adapted to soils contaminated with heavy metals from mine tailings (e.g., Antonovics 1968, 1972; McNeilly, 1968; McNeilly & Antonovics, 1968). These are particularly desirable model systems because there is divergent selection at the local level, divergence has been recent (Macnair & Gardner, 1998), and it is possible to assess gene flow between the two types. These are quite clearly p-d situations at the population, if not the species, level.

Antonovics (2006) has provided a brief review of earlier work and updated the situation for the grass species *Anthoxanthum odoratum* L. (sweet vernal grass) found growing on soil contaminated with lead and zinc from mine tailings. It is more parsimonious to assume that the tolerant plants have been selected from within non-tolerant populations at the locality where they now occur rather than to hypothesize an allopatric phase (Macnair & Gardner, 1998), and the recent development of the contaminated soils suggests that divergence has occurred rapidly (Antonovics, 2006). Spatial separation of populations, though at a local scale, is maintained by the two edaphic ecotypes. Thus, reduced gene flow between the two populations is a byproduct of adaptive divergence. Further, plants on the mine tailings flower earlier than those off the tailings, though there is some overlap, and this pattern has been maintained for over 40 years (McNeilly & Antonovics, 1968; Antonovics & Bradshaw, 1970; Antonovics, 2006). It is important to emphasize that the flowering time differences have not been maintained simply because the same plants have been present during the past 40 years; the half-life of plants on the mine is

less than two years (Antonovics, 1972). McNeilly & Antonovics (1968) demonstrated that flowering time has a genetic basis, and is not significantly correlated with metal tolerance. Calculation of overlap in flowering time between the metal and normal soils indicated a 40% reduction in mating between the two types relative to mating between plants of the same type. Another factor potentially reducing gene flow between tolerant and nontolerant types is the greater self-fertility of the tolerant plants (Antonovics, 1968). Antonovics & Bradshaw (1970) found several small phenotypic differences between tolerant and nontolerant plants and Antonovics (2006: 36) suggested that, “Such differences are often used in species level taxonomic characterizations.” It would be possible to determine selection against immigrants into each of the habitats, and thus obtain an estimate of the dynamics of the system. The mechanisms of heavy metal accumulation and tolerance have been extensively studied, and represent an excellent system for detailed studies at the genetic-genomic levels (e.g., Roossens & al., 2008; Verbruggen & al., 2009).

More than 30 years ago, Antonovics (1976: 241) emphasized that, “The distinction between ‘ecological time’ and ‘evolutionary time’ is artificial and misleading”; similar views have been stated more recently (e.g., Carroll & al., 2007). Recent reviews have indicated that the speed of speciation is unknown (Rieseberg & Willis, 2007), but there is little doubt that speciation can commence in generations, that is, some reproductive barriers may evolve (e.g., Hendry & al., 2007). There is still the question, however, of whether it goes to completion. Only after it is known how much and what kind of gene flow is reduced by early stages of divergence, and the strength of divergent selection (Nosil & al., 2008; Sobel & al., 2010) can there be any estimate of how far along the spectrum of speciation the initial barriers place two entities.

Assuming that one or more isolating barriers can be identified, a next step (or next several steps) is to elucidate the genetic architecture of barriers to gene flow. For example, genome scans examining many different loci for populations or species differing in phenotypic, physiological or other traits may reveal “outlier” markers, that is, those showing high levels of divergence between plants with the different traits (e.g., Savolainen & al., 2006; Scotti-Saintagne & al., 2004). These outlier loci are of particular interest because they may be linked to loci that affect alternative phenotypic traits (Mitchell-Olds & Schmidt, 2006; Via, 2009). A next step would be to find the regions of the genome associated with the variation (e.g., quantitative trait loci) (Mitchell-Olds & Schmidt, 2006), and the ultimate goals are to not only find and characterize candidate loci but to demonstrate that they “make a difference” for adaptation in different environments. Needless to say these are huge tasks, even with so-called model organisms (Weigel & Nordborg, 2005; Mitchell-Olds & Schmitt, 2006). The “magnifying glass” approach is best for understanding, at the finest genetic scale, the polymorphisms that are factors important in the initial reduction in gene flow.

The “spyglass” and “magnifying glass” approaches do, however, each have tradeoffs in studies of speciation, while each provides insights. With the former approach, the process

has presumably gone to completion whereas in the latter speciation is not complete, and it cannot be determined whether it will go to completion and result in two distinct evolutionary lineages. With the “spyglass” approach, there is no doubt that the two entities should be recognized as distinct species, with speciation then complete, but there is the aforementioned problem of the accumulation of differences subsequent to the completion of speciation (Sobel & al., 2010). It is obvious that the neither approach by itself is totally satisfying for studying speciation. However, if using the “magnifying glass” approach in different studies revealed similar isolating barriers, it would suggest that they frequently evolve during early stages of speciation.

While it can never be known for certain, it may well be that the p-d model of speciation is more common in plant speciation than has been generally recognized. Although it is a big challenge to detect the best candidates for study (Gottlieb, 2003), a much larger task is carrying out the broad yet detailed genetic and ecological studies necessary to obtain greater insight into the processes by which divergence occurs. Highly differentiated sister species may be easily recognized, but they offer limited promise for elucidating the processes that produced them. While the time and effort required to identify and study p-d species pairs in depth are considerable, such efforts will be rewarded with refined insights into plant speciation.

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