The Ecological Genetics of Homoploid Hybrid Speciation

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Abstract

Our understanding of homoploid hybrid speciation has advanced substantially since this mechanism of species formation was codified 50 years ago. Early theory and research focused almost exclusively on the importance of chromosomal rearrangements, but it later became evident that natural selection, specifically ecological selection, might play a major role as well. In light of this recent shift, we present an evaluation of ecology's role in homoploid hybrid speciation, with an emphasis on the genetics underlying ecological components of the speciation process. We briefly review new theoretical developments related to the ecology of homoploid hybrid speciation; propose a set of explicit, testable questions that must be answered to verify the role of ecological selection in homoploid hybrid speciation; discuss published work with reference to these questions; and also report new data supporting the importance of ecological selection in the origin of the homoploid hybrid sunflower species *Helianthus deserticola*. Overall, theory and empirical evidence gathered to date suggest that ecological selection is a major factor promoting homoploid hybrid speciation, with the strongest evidence coming from genetic studies.

Ecological Speciation: A Historical Perspective

It is often lamented that ecology has been overlooked in studies of speciation (Lewontin 1997; Morell 1999; Schemske 2000). This is a curious claim given that Darwin's focus was primarily on ecological divergence and that both Dobzhansky (1937) and Mayr (1942) argued that adaptation to different environments led to speciation. Early botanical students of speciation put an even greater stress on ecology. For example, Stebbins (1950, p. 199) wrote, "By far the most common type of isolation in both the plant and the animal kingdom is that resulting from the existence of related types in different geographical regions which differ in the prevailing climatic and edaphic conditions." Likewise, Clausen's (1951) monograph describes how ecological races are transformed into species and defines species in terms of their physiologic-genetic properties.

Although it may be that Dobzhansky and Mayr simply played lip service to ecology (Coyne and Orr 2004), this argument doesn't hold for their botanical counterparts. Stanford University's influential team of Clausen, Keck, and Hiesey pioneered the field of ecological genetics (e.g., Clausen et al. 1947), Verne Grant's (1949) empirical work focused on pollinator-mediated isolation, and Edgar Anderson (1948) emphasized the role of habitat disturbance in the breakdown of reproductive barriers and establishment of hybrids. Even Stebbins, whose empirical work was primarily

cytological, published several papers on ecological aspects of speciation (e.g., Stebbins 1942, 1952).

It is easier to make the case that ecology was neglected in the latter half of the twentieth century, even in plants. Population geneticists and systematists were mesmerized by new molecular genetic tools that allowed genetic variability to be measured and the history of populations and species to be reconstructed, whereas ecologists either were not interested in speciation or were preoccupied with measuring selection in natural populations. More generally, the field of speciation was sidetracked by theoretical arguments favoring nonecological causes of speciation, such as population bottlenecks and genetic drift. As a consequence, investigations of the ecology of speciation stagnated along with other process-oriented studies of speciation.

Fortunately, the situation has changed dramatically over the past decade. A new cadre of ecologists has become interested in speciation, influential models of ecological speciation have been developed (e.g., Doebeli and Dieckmann 2003), and explicit tests have been put forward to distinguish ecological speciation from other speciational mechanisms (Schluter 2001). However, these tests were developed to assess the role of ecological selection in primary speciation. A comparable experimental framework for testing the importance of ecological selection in homoploid hybrid speciation has not yet been established, although several requirements have been suggested ad hoc in recent papers (Gross et al. 2004; Lexer et al. 2003b; Rieseberg et al.

Table 1. Characteristics of reticulate modes of speciation (cf. Figure 1).

	Chromosomal changes	Initial form of reproductive isolation	Genetic mechanism(s) underpinning initial isolation	Proximate cause(s) of isolation
Allopolyploidy	Genome duplication	Intrinsic postzygotic	Ploidy differences; mutation	Genetic drift
Homoploid hybrid speciation[a]				
1. Recombinational model	Rearrangements; recombination of parental rearrangements	Intrinsic postzygotic	New chromosomal combinations via hybridization	Genetic drift; fertility selection
2. External isolation model	None	Ecological prezygotic; extrinsic postzygotic	New genetic combinations via hybridization	Divergent natural selection (includes ecological selection)

[a] Note that many cases of homoploid hybrid speciation incorporate aspects of both the recombinational and external isolation models.

2003). (See Table 1 and Figure 1 for characteristics of reticulate speciation.)

The objectives of the present article are to (1) briefly review new theoretical developments related to the ecology of homoploid hybrid speciation; (2) propose a set of explicit, testable questions designed to verify the role of ecological selection in the process; (3) discuss published work on the ecology of homoploid hybrid speciation with reference to these tests; and (4) report new data supporting the importance of ecological selection in the origin of the homoploid hybrid sunflower species *Helianthus deserticola*.

Homoploid Hybrid Speciation: Theory

During homoploid hybrid speciation, a stable, fertile, and reproductively isolated hybrid derivative arises without a change in chromosomal number. Reproductive isolation is thought to occur through rapid chromosomal reorganization, ecological divergence, and/or spatial isolation (Rieseberg 1997). Early verbal models of the process emphasized the evolution of chromosomal sterility barriers, which were thought to arise through the sorting of chromosomal rearrangements that differentiated the parental species (Grant 1958; Stebbins 1957). This chromosomal model, referred to as "recombinational speciation" by Grant (1981), has received the most attention in subsequent study. However, Grant also noted that a hybrid lineage could arise through "the segregation of a new type isolated by external barriers" (1981, p. 243). Templeton (1981) suggested that both forms of isolation were likely to be important, with chromosomal isolation preventing extinction through genetic assimilation and ecological divergence precluding extirpation through competition.
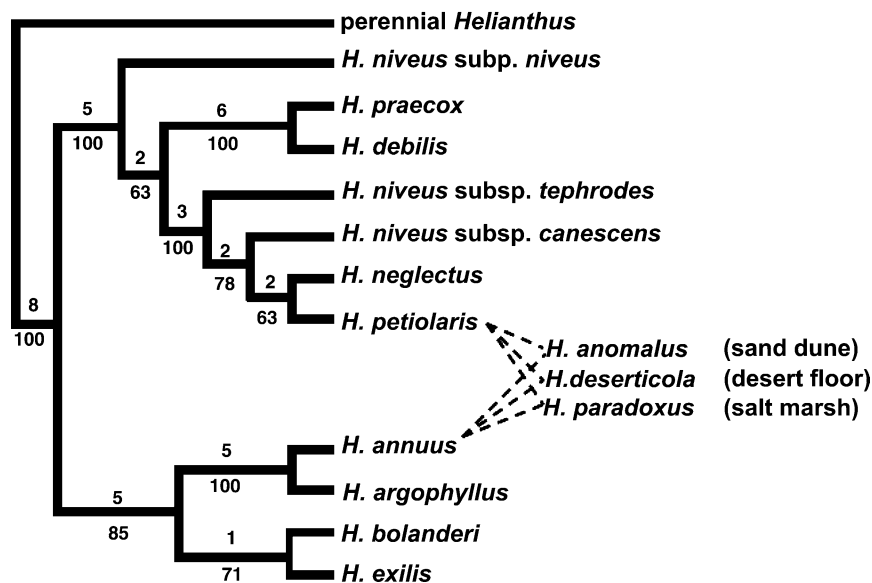
Figure 1. Example of a phylogenetic tree showing reticulation due to homoploid hybrid speciation. Phylogenetic tree for *Helianthus* section, *Helianthus* based on combined chloroplast DNA and nuclear ribosomal DNA data (Rieseberg 1991). The number of mutations are given above and bootstrap percentages below each branch. Dashed lines indicate parentage of homoploid hybrid species.

The first mathematical treatment of homoploid hybrid speciation was a computer simulation of the recombinational model (McCarthy et al. 1995). Because speciation failed to occur at an appreciable rate by the sorting of chromosomal rearrangements alone, McCarthy et al. simulated ecological selection by arbitrarily assigning a fitness advantage to some hybrid genotypes. When the fitness advantage was large, hybrid speciation occurred at an appreciable rate. However, the McCarthy et al. model describes a special case of hybrid speciation in which the new hybrid lineage replaces its parental species rather than coexisting with them. Also, although it is theoretically possible that a hybrid genotype might be fitter than its parents in parental habitats (Barton 2001), superior hybrid fitness in a new habitat is considerably more plausible (Arnold 1997; Rieseberg 1997).

The role of ecological selection was explored explicitly by Buerkle et al. (2000) in a model featuring habitats for both of the parental species and a third unoccupied, ecologically divergent habitat available for colonization. This spatial model demonstrated that the frequency of hybrid speciation increased with increasing strength of ecological selection. As in the McCarthy et al. (1995) model, the sorting of chromosomal rearrangements alone was unlikely to lead to speciation. Although strong ecological selection was sufficient for hybrid speciation, the evolutionary independence of the new hybrid lineage was increased by chromosomal isolation as predicted by Templeton (1981). Ecological selection thus emerged as an important factor for the origin of homoploid hybrid species, at least theoretically.

This model was subsequently modified by removing the unoccupied habitat, leaving only the two parental habitats available (Buerkle et al. 2003). Under these conditions, homoploid hybrid speciation was rare. When it did occur, the new hybrid populations were generally small, and unstable, and appeared to represent a transitory state prior to the genetic assimilation of one species by the other. Clearly, these results indicate that the availability and colonization of a novel habitat are crucial to the origin of homoploid hybrid species.

Finally, there has recently been a more thorough consideration of the origin of traits that allow hybrids adapt to new environments and thereby diverge from their parental species ecologically (Barton 2001; Ellstrand and Schierenbeck 2000; Schwarzbach et al. 2001). Hybrids may have intermediate trait values, combine traits from both parents, and/or exhibit extreme trait values. All three possibilities might allow for high fitness of hybrids in a new environment. For example, an intermediate hybrid might have higher fitness in an environment that is intermediate compared to parental habitats (Anderson 1948), whereas a novel combination of parental traits might be advantageous in a habitat that has aspects of both parental environments. However, some homoploid hybrid species are also found in extreme habitats, a transition that likely requires the acquisition of novel, perhaps extreme traits (Gross et al. 2004; Lexer et al. 2003a,b; Rieseberg et al. 1999, 2003; Schwarzbach et al. 2001). Hybridization provides a means by which this could occur because extreme or transgressive trait values are common in both natural hybrid populations and controlled crosses

(Rieseberg et al. 1999) and appear to mostly result from the complementary action of alleles from both parents—that is, the "stacking" of alleles with the same directional effect on a given trait (deVincente and Tanksley 1993).

Tests of Ecological Speciation

Schluter (2001) has suggested several tests to distinguish primary ecological speciation from other speciation mechanisms, such as ordinary genetic drift, drift resulting from population bottlenecks, divergence accruing from parallel selection, and speciation by polyploidy. These tests include (1) a correlation between the strength of divergent selection and rate of evolution of reproductive barriers, (2) evidence of ecological selection against hybrids, (3) evidence of divergent natural selection on phenotypic traits or specific genes, and (4) parallel speciation.

The situation for homoploid hybrid speciation is more complex, however, because we need to show not only that ecological divergence in the new hybrid lineage contributes to isolation but also that the divergence arose early in the hybrid speciation process. If most ecological changes accrue after hybrid speciation, niche divergence could be incidental to speciation. Also, it is important to understand the origins of the ecological divergence in homoploid hybrid species. That is, we need to demonstrate that hybridization is both sufficient and necessary for generating the specific trait combinations and trait values that are the basis for ecological divergence and reproductive isolation. This would also imply that ecological divergence arose early in the speciation process.

Given these considerations, we suggest the that a careful evaluation of the following questions would verify the role of ecological divergence in homoploid hybrid speciation. Some of these are self-evident and can largely be inferred from descriptive analyses of ecogeographic distributions, whereas others will require explicit experimentation.

1. Are the homoploid hybrid species ecologically divergent from their parents?
2. Does ecological divergence contribute to reproductive isolation?
3. Can the new combinations of traits and genes responsible for ecological divergence be generated via hybridization (as opposed to the gradual accumulation of mutations)?
4. Are traits and genes responsible for ecological divergence under divergent natural selection in the habitat of the hybrid species?
5. Is selection strong enough to allow divergence in the face of gene flow (given that homoploid hybrid speciation must be initiated in sympatry or parapatry with its parental species)?
6. Is there evidence of parallel hybrid speciation?

Are the Homoploid Hybrid Species Ecologically Divergent from their Parents?

Although homoploid hybrid speciation is frequently proposed as an explanation for morphological intermediacy,

Table 2. Confirmed and potential homoploid hybrid species, description of organism type, mode of ecological divergence from parental species, documentation of multiple origins, and references.

Taxon	Description	Ecological divergence	Multiple origins	Reference
Alcyonium hibernicum[b]	marine invertebrate	habitat	unknown	McFadden and Hutchinson 2004
Argyranthemum sundingii[a]	annual herb	habitat	yes	Brochmann et al. 2000
Arisaema ehimense[b]	perennial herb	habitat and temporal	unknown	Maki and Murata 2001
Armeria villosa carratracensis[b]	perennial herb	habitat	unknown	Feliner et al. 2002
Ceanothus species[b]	perennial shrub	habitat	unknown	Hardig et al. 2002
Daphnia mendotae[a]	freshwater invertebrate	habitat	unknown	Taylor et al. 1996
Encelia virginensis[b]	perennial shrub	habitat	unknown	Allan et al. 1997
Gila seminude[a]	freshwater fish	habitat	unknown	DeMarais et al. 1992
Helianthus anomalus[a]	annual herb	habitat	yes	Rieseberg 1991
Helianthus deserticola[a]	annual herb	habitat	yes	Rieseberg 1991
Helianthus paradoxus[a]	annual herb	habitat and temporal	no	Rieseberg 1991, Rieseberg et al. 1991
Hyobanche glabrata[b]	parasitic herb	habitat and pollinator	unknown	Wolfe and Randle 2001
Iris nelsonii[a]	annual herb	habitat	unknown	Arnold 1993
Peaonia species group[a]	perennial herb	habitat	unknown	Ferguson and Sang 2001; Sang et al. 1995, 1997; Sang and Zhang 1999
Penstemon clevelandii[a]	annual herb	pollinator	unknown	Wolfe et al. 1998
Pinus desata[a]	perennial tree	habitat	yes	Wang et al. 2001
Schistosoma sinensium[b]	parasitic invertebrate	habitat	unknown	Hirai et al. 2000
Senecio eboracensis[c]	annual herb	temporal and pollinator	no	Lowe and Abbott 2004
Stephanomeria diegensis[a]	annual herb	habitat	unknown	Gallez and Gottlieb 1982

[a] Known cases of homoploid hybrid speciation confirmed with molecular markers.

[b] Potential but unconfirmed cases of homoploid hybrid speciation.

[c] Confirmed hybrid speciation event, only one parent with the same ploidy level as hybrid species.

there are still only a handful of instances in which these hypotheses have been rigorously confirmed using molecular markers (reviewed by Coyne and Orr 2004; Rieseberg 1997). The majority of these studies involve plants, but several examples have recently been reported from invertebrates and fish. In all cases, there is some measure of ecological divergence between the hybrid and its progenitors, although the thoroughness with which this has been documented varies. In most instances, the only information derives from brief descriptions of the habitat that hybrid neospecies occupies (see later discussion), although in few cases common garden and transplant experiments have been performed. Ecological divergence in a majority of known homoploid hybrid species would be unlikely if speciation was driven by intrinsic factors, such as chromosomal rearrangements. Overall, the consistent pattern of ecological divergence described conforms well to expectations if ecological divergence is important in promoting homoploid hybrid speciation (Table 2).

The first homoploid hybrid species to be confirmed with molecular markers was *Stephanomeria diegensis*, a derivative of *S. exigua* and *S. virgata* (Gallez and Gottlieb 1982). The hybrid species is found mainly in disturbed or pioneer habitats in southern California, a range that is restricted in both area and elevation when compared to those of its progenitors, which have extensive ranges in southwestern North America. *S. exigua* is generally found in sandy soils occupied by sagebrush or creosote bush, whereas *S. virgata* is common in chaparral openings and dry sandy hills.

Iris nelsonii also has a long history in the hybrid literature (e.g., Randolph 1966), and its diploid hybrid ancestry was confirmed in 1993 (Arnold). There are three parental species all found in Louisiana: *I. fulva* inhabits the shady, shallow water of bayou margins; *I. hexagona* is found in sunny, deeper swamp water; and *I. brevicaulis* occurs in much drier pastures and forests. In this system, the hybrid species occupies a divergent habitat that combines features from habitats of the parental species, and is found in the shady, deep water of cypress swamps.

Several hybrid species have been identified in the widespread *Peaonia* complex of Europe and Asia (Ferguson and Sang 2001; Sang et al. 1995, 1997; Sang and Zhang 1999). However, information on the ecology of the hybrid and parental *Peaonia* species is not generally reported. Biogeographic distributions are known for many of the species, and the hybrid derivatives are often allopatric with respect to their progenitors. Interestingly, European populations of the progenitors appear to have been displaced by the hybrid species, perhaps due to the superior ability of the hybrids to adapt to Pleistocene climactic changes in Europe (Sang et al. 1995).

The hybrid species *Argyranthemum sundingii* (Brochmann et al. 2000) exhibits a more classic kind of ecological divergence, occurring at an intermediate elevation and moisture gradient relative to the parents (Tenerife, Canary Islands). Greenhouse experiments have identified several phenotypic differences that are associated with habitat differentiation in the hybrid neospecies and shown that these differences are heritable.

The origin of *Penstemon clevelandii* from *P. centranthifolius* and *P. spectabilis* is of interest because reproductive isolation of the hybrid neospecies may have been achieved by selection for a divergent pollination syndrome (Straw 1956; Wolfe et al. 1998). The hybrid species is bee- and hummingbird-pollinated, with magenta colored, semi-inflated flowers and occurs in canyons and road cuts in granitic soils of southern California and northern Baja. *Penstemon spectabilis* is wasp-pollinated with lavender, fully inflated flowers and is a pioneer species of disturbed habitats in southern California and northern Baja, whereas *P. centranthifolius* is hummingbird-pollinated with red, tubular flowers and is typically found in sandy washes and roadsides over the same general range as *P. spectabilis*.

Senecio eboracensis ($2n = 40$) is unique relative to many other hybrid species; it has the same ploidy as the parental species *S. vulgaris* ($2n = 40$) but double the ploidy of the second parental species *S. squalidus* ($2n = 20$) (Abbott and Lowe 2004). Thus it is neither a homoploid nor a polyploid hybrid species according to the strict definitions of these terms. Nonetheless, it is important to consider this example due to the fact that reproductive isolation between the *S. eboracensis* and *S. vulgaris* must be based on mechanisms other than ploidy changes. The hybrid species shows only slight ecological divergence relative to *S. vulgaris* and the two taxa occur sympatrically in the British Isles, sometimes only meters away from each other. However, the species differ slightly in seed germination, seedling over wintering, flowering time, and floral morphology (Lowe and Abbott 2004).

There are two genera in which diploid hybrid species clearly inhabit environments that are extreme relative to the parental species: *Pinus* and *Helianthus*. *Pinus densata*, which is derived from hybridization between *P. yunnanensis* and *P. tabulaeformis*, inhabits a high mountain environment where the parental species are apparently unable to grow (Wang et al. 2001). The hybrid species occurs between 2700 and 4200 m above sea level on the Tibetan Plateau, whereas the parental species occur from sea level to 3100 m.

The genus *Helianthus* (the North American sunflower) contains three hybrid species that occur in three widely divergent habitats (Heiser 1947; Heiser et al. 1969; Rogers et al. 1982), despite sharing the same two parents (Figure 1) (Rieseberg 1991). Both parental species are widely distributed across the United States; *H. annuus* is found in mesic, clay-based soils and *H. petiolaris* occurs in dryer, sandier soils. The hybrid species, in contrast, have restricted ranges in the southwestern United States; *H. anomalus* is found on active sand dunes, *H. deserticola* occupies xeric habitats in the Great Basin Desert, and *H. paradoxus* inhabits desert salt marshes. Greenhouse comparisons have identified a suite of traits that differentiate each taxon from its parental species and that seem likely to confer a fitness advantage to the homoploid hybrid species in its native habitat (Rosenthal et al. 2002; Schwarzbach et al. 2001). For example, *H. deserticola* has early flowering and small leaves compared to the parental species; both traits are typical of desert annuals. Transplant experiments indicate that the parental species fail to survive in *H. paradoxus* habitat (Lexer et al. 2003b) may be as fit as the hybrid taxon in the *H. anomalus* habitat (Ludwig et al. in press), and exhibit equivalent or greater fitness than the hybrid species in *H. deserticola* habitat (Gross et al. 2004). However, the parental species showed reduced or no emergence in the *H. anomalus* habitat during two following experiments, suggesting that their exclusion may be due to failure in germination and early growth. Patterns of seedling emergence and survival from similar experiments in the *H. deserticola* habitat remain unclear and will require further investigation (Ludwig and Donovan personal communication).

The best example of hybrid speciation in animals comes from the fish genus *Gila*, native to the southwestern United States (DeMarais et al. 1992). The parental species, *G. robusta robusta* and *G. elegans*, are found sympatrically in the large Colorado River, whereas the hybrid species, *G. seminude*, is found only in the Virgin River, a moderately sized tributary of the Colorado. Interestingly, the parental species are never found in the Virgin River, although there are no known physical barriers that would impede their migration.

Homoploid hybrid speciation also has been proposed for the water flea *Daphnia mendotae*, which occurs in slightly warmer water and shows less vertical migration than one if its sympatric progenitors, *D. dentifera* (Dudycha 2003; Taylor et al. 1996). Hybrid speciation may also have occurred in the soft coral genus *Alcyonium*, where the hybrid species is almost completely allopatric with its progenitors (McFadden and Hutchinson 2004).

Finally, there are multiple proposed cases of homoploid hybrid speciation that have proven difficult to verify even with molecular markers, including species in the genera *Armeria* (Feliner et al. 2002), *Arisaema* (Maki and Murata 2001), *Ceanothus* (Hardig et al. 2002), *Encelia* (Allan et al. 1997), *Hyobanche* (Wolfe and Randle 2001), and *Schistosoma* (Hirai et al. 2000). These taxa may provide additional support for ecological divergence if their hybrid ancestry can be confirmed.

Does Ecological Divergence Contribute to Reproductive Isolation?

Ecological selection may contribute to reproductive isolation via habitat, floral/pollinator, and temporal divergence. All three types of divergence are potentially important in promoting homoploid hybrid speciation, but most research to date has focused on habitat divergence, which can lead to spatial isolation (a prezygotic barrier) and reduced fitness of hybrids in parental habitats (a postzygotic barrier). As noted by Coyne and Orr (2004), however, habitat divergence does not necessarily cause reproductive isolation. For example, plants might partition water usage in different ways but still flower simultaneously and hybridize readily. Do the ecological differences documented for various homoploid hybrid species actually contribute to reproductive isolation? Not surprisingly, this question remains to be investigated in many systems, but some answers are emerging.

Cases where hybrid species and their progenitors are partially or completely allopatric due to habitat divergence come the closest to satisfying the requirement of reproductive isolation. This group includes *Peaonia*, *Pinus*, and *Gila* on a macrospatial scale and *Stephanomeria*, *Iris*, and *Argyranthemum* on a microspatial scale. *Helianthus* is on the border between these extremes; the parental species overlap the hybrid taxa on a broad scale but generally occur several kilometers from each other at a given site. This spatial isolation appears to be an effective barrier to hybridization. For example, no hybridization has been detected between the ancient hybrid sunflower species and their progenitors, despite extensive fieldwork (Gross et al. 2003; Heiser et al. 1969; Rogers et al. 1982; Schwarzbach and Rieseberg 2002; Welch and Rieseberg 2002). No attempts have been made to assess whether synthetic hybrids between a homoploid hybrid species and its progenitors would have reduced fitness in hybrid or parental habitats. However, the transplant experiments conducted for sunflowers (described earlier) provide a possible explanation for why the parental species fail to colonize the habitats of their hybrid derivatives.

Both temporal and pollinator divergence are thought to contribute to reproductive isolation between *S. eboracensis* and *S. vulgaris*. However, a thorough investigation of reproductive isolation in this system suggests that these ecological factors are probably less important in preventing hybridization than the fact that both species are predominantly self-fertilizing (Abbott and Lowe 2004; Lowe and Abbott 2004).

Penstemon offers a possible example of hybrid speciation dependant on solely ecological isolating barriers (floral/pollinator isolation and possibly microspatial habitat isolation). The two parental species are pollinated by different animals, and the hybrid species is thought to exploit a third (Straw 1956). Although anecdotally compelling, precise studies quantifying the degree of pollinator discrimination have not been conducted for the hybrid species, and hybridization between the derivative and parental species has been suggested (Chari and Wilson 2001; Wilson and Valenzuela 2002). Temporal divergence seems to be important in sunflower speciation, where *Helianthus paradoxus* flowers substantially later than both parental species (Heiser et al. 1969). However, the effectiveness of this temporal barrier to hybridization has not been quantified.

Can the New Combinations of Traits and Genes Responsible for Niche Divergence Be Generated via Hybridization?

One feature of homoploid hybrid speciation that renders it unique relative to most other modes of speciation is the potential for experimental manipulation. Synthetic hybrids between parental taxa can effectively serve as approximations of the ancestral genotype of the hybrid species, in contrast to the situation for most modern species, where the progenitor is unknown or extinct. It is straightforward, therefore, to test whether the traits or trait combinations

required for ecological divergence could have been generated by hybridization. The alternative possibility is that the ecological divergence was mostly achieved after speciation through the gradual accumulation of new mutations. As far as we are aware, these experiments have only been carried in two systems: *Argyranthemum* and *Helianthus*.

Brochman et al. (2000) created synthetic F_2 hybrids between *Argyranthemum broussonetii* and *A. frutescens*, the putative parents of the homoploid hybrid species *A. sundingii*, and grew them in the greenhouse along with the pure species. The hybrid species was intermediate between the two parental species for all nine measured traits, though usually closer to *A. frutescens* than *A. broussonetii*. The synthetic hybrids overlapped the phenotypic range of the homoploid hybrid species for eight of nine traits, showing that the traits could be produced easily via hybridization. The authors suggested that *A. sundingii* was likely generated via a back-cross toward *A. frutescens*, so a BC_1 or BC_2 population would presumably have yielded an even greater overlap in phenotype.

In sunflowers, hybrid species exhibit many traits that are extreme relative to parental species, rather than being exclusively intermediate (Rosenthal et al. 2002; Schwarzbach et al. 2001). Multiple experiments have been conducted in the greenhouse and in the natural habitats of the three hybrid species to evaluate the potential for recreating these extreme traits in synthetic hybrids (second generation backcrosses toward both parents; hereafter BC_2Ann and BC_2Pet). Analyses of 40 traits in greenhouse-grown plants revealed that the ancient hybrid species' trait values could be fully recovered in synthetic hybrids for all extreme traits in *H. anomalus* and *H. deserticola* and all but three extreme traits in *H. paradoxus* (Rieseberg et al. 2003; Rosenthal personal communication).

For the field experiments, which are more relevant ecologically, individuals of *H. annuus*, *H. petiolaris*, and one of the hybrid species were planted in that hybrid species' native habitat along with the synthetic hybrids. However, the results were similar. *Helianthus anomalus*, a desert sand dune endemic, was positively transgressive for leaf succulence and negatively transgressive for leaf nitrogen in its natural habitat (Ludwig et al. in press). Synthetic hybrids overlapped *H. anomalus* for both traits, although for leaf succulence the overlap was slight. *Helianthus deserticola*, found in the xeric environment of the Great Basin Desert, was negatively transgressive for leaf area, stem diameter, and flowering date. As in the greenhouse studies, extreme values for all three traits were recovered in synthetic hybrids (Gross et al. 2004). *Helianthus paradoxus* inhabits highly saline desert marshes and field-grown plants were transgressive for five traits: sulfur, calcium, and boron content; leaf shape; and leaf succulence (Lexer et al. 2003b). However, unlike the greenhouse experiments, synthetic hybrids overlapped the *H. paradoxus* phenotype for all five traits despite the fact that only BC_2Pet hybrids were tested. These experiments show that extreme or transgressive phenotypes for ecologically relevant traits can be re-created via hybridization.

The sunflower work was extended to determine the genetic mechanism underlying the generation of extreme trait values in the synthetic hybrids and to ask if the same mechanism was responsible for production of similar traits during the origin of the ancient hybrid species more than 60,000 years ago (Gross et al. 2003; Schwarzbach and Rieseberg 2002; Welch and Rieseberg 2002). Mapping of quantitative trait loci (QTLs) in the greenhouse-grown synthetic hybrids described earlier (Lexer et al. in press; Rieseberg et al. 2003), as well as those raised in the *H. paradoxus* habitat (Lexer et al. 2003a) revealed that extreme or transgressive trait values were almost certainly generated by complementary gene action. This makes sense given that complementary gene action has proven to be the primary cause of transgressive segregation in crop hybrids (e.g., deVincente and Tanksley 1993). Furthermore, it was shown that closely linked or pleiotropic QTLs generally had effects in same direction with respect to the phenotypes of the hybrid species. Given these favorable genetic correlations, it should be feasible to generate the complex, multitrait phenotypes of the ancient hybrid species after only a few generations of recombination.

Despite evidence of feasibility, the possibility remains that phenotypic/ecological divergence was mostly achieved after speciation through the gradual accumulation of new mutations and that the detected hybridization was co-incidental to the process. This objection is laid to rest, however, by comparisons of the QTL mapping data with detailed genetic maps of the diploid hybrid species (Rieseberg et al. 2003). If the phenotypes of the diploid hybrid species arose through complementary gene action as hypothesized, then we should be able to predict the genomic composition of the hybrid species from the QTL mapping data. Parentage of 71.8% of markers in *H. paradoxus*, 75.6% of markers in *H. anomalus*, and 79.3% of markers in *H. deserticola* was predicted correctly ($p \ll .0001$ for all comparisons), indicating that hybridization and complementary gene action did contribute to the phenotypic and ecological divergence of ancient sunflower hybrids.

Are Traits and Genes Responsible for Ecological Divergence under Divergent Natural Selection in the Habitat of the Hybrid Species?

Synthetic hybrids are useful not only for evaluating trait expression but also for re-creating the selective pressures acting on hybrid neospecies at their origin. If ecological selection was important in speciation and shaped the phenotype and genotype of the hybrid species, then selection acting on synthetic hybrids in the field should favor the trait values found in present-day populations of the ancient hybrid species. Such investigations have only been carried out in *Helianthus* to date; we report the results here.

Parallel phenotypic selection experiments (Lande and Arnold 1983) have been carried out in the habitat of all three hybrid sunflower species to estimate the strength and direction ecological selection acting on early generation hybrids in the field. Selection differentials and gradients were calculated for synthetic BC_2 hybrids (described earlier), with a focus on traits that are transgressive in the hybrid species today. Overall, these field experiments have shown that many of the extreme traits found in the ancient hybrid species could have arisen via divergent ecological selection acting on transgressive hybrids growing in novel habitats. These traits include high leaf succulence in *H. anomalus* (Ludwig et al. in press), small leaves and early flowering in *H. deserticola* (Gross et al. 2004), and high leaf succulence, high calcium content, and low uptake of toxic elements in *H. paradoxus* (Lexer et al. 2003b).

However, these studies failed to detect significant selection on several traits, and in several instances selection was not in the predicted direction. It may be that these contradictory results are an artifact of the restricted spatial and temporal scale of the selection experiments. Given that selection fluctuates over both time and space (Grant and Grant 2002), it is not surprising that selective pressures measured over a single growing season are not fully consistent with predictions based on current phenotypes of the hybrid taxa. Despite these shortcomings, the experiments successfully demonstrate that divergent ecological selection may allow transgressive sunflower hybrids to colonize new habitats and thereby achieve some degree of habitat isolation from their progenitors.

Is Selection Strong Enough to Allow Divergence in the Face of Gene Flow?

Because hybrids are necessarily produced in proximity with one or both of their parental species, homoploid hybrid speciation represents a kind of speciation with gene flow, which requires that the strength of selection on individual loci, *s*, exceed the migration rate, *m*. The fact that introgression is a far more frequent outcome of hybridization than speciation implies either that this condition is rarely met or that open niches are rarely available for colonization by hybrids (Arnold 1997; Buerkle et al. 2000; Rieseberg and Wendel 1993). In those cases where hybrid speciation has occurred, it is necessary to determine whether ecological selection alone would have been strong enough to drive divergence or whether migration rates had to be reduced by some other form of isolation (e.g., chromosomal or spatial isolation) before ecological divergence could occur.

The parameter *m* can be estimated fairly easily from population-level surveys of molecular markers, such as microsatellites. Approximating *s* is more challenging, because loci controlling a trait of interest must identified and relative fitness must be estimated for individuals that segregate for parental species' alleles at that locus. This combination of fieldwork and genetic mapping is rare, so the relationship between migration and selection has only been calculated for hybrid taxa in the genus *Helianthus*.

The phenotypic selection study in *H. paradoxus* was extended to the genetic level via QTL analyses of the population of synthetic hybrids grown in salt marsh habitat. Selection coefficients on individual salt tolerance QTLs in the *H. paradoxus* habitat were large (+0.126, −0.084, and

Table 3. Putative QTL linkage groups, interval markers, positions, percent phenotypic variance explained (PVE), additive effects, proxy microsatellite marker, and selection coefficient (*s*) for two traits under selection in a second generation backcross (BC$_2$) population of *Helianthus annuus* × *H. petiolaris* toward *H. petiolaris* grown in the habitat of *H. deserticola*.

Trait	Linkage group	Interval markers	Position (cM)	PVE (%)	Additive effect	LRS	Marker[a]	*s*
Stem diameter	7	ORS702b-ORS163	87–118	3	−1.04	8.7[b]	ORS163	−0.027
Leaf width	10	ORS591-ORS256	0–11	4	+3.57	12.7[c]	ORS256	−0.050
	10	ORS3-ORS691	26–28	4	+3.17	8.9[c]	ORS3	−0.031

[a] Marker used as a proxy for QTL to calculate value of *s*.

[b] LRS value marginally statistically significant, *p* = .065 based on 1,000 permutations of the data.

[c] LRS values exceed the level for statistical significance based on 1,000 permutations of the data.

−0.094) (Lexer et al. 2003a). Current estimates of the parameter N_em for annual sunflowers are consistently less than or equal to 1. Thus, a slight excess of 12 individuals would be sufficient to allow selection on the founding population of the *H. paradoxus* lineage to overcome the homogenizing effects of gene flow. The analysis also revealed that QTLs contributing to increased fitness in the salt marsh were derived from both parental species, as predicted by the complementary gene action model if hybridization facilitated adaptation to this new environment.

The phenotypic selection study on the desert floor was likewise expanded to the genetic level, focusing on the BC$_2$Pet cross due to its similarity to the *H. deserticola* phenotype (Gross unpublished data). The study in the *H. deserticola* habitat took advantage of the large number of QTLs controlling morphological, physiological, and life history traits that had been documented for the BC$_2$Pet cross in a previous greenhouse study (Rieseberg et al. 2003), rather than constructing a new QTL map. Each chromosomal interval containing a QTL for a trait of interest in the greenhouse was considered as a candidate QTL region in the field population, and the BC$_2$Pet field population was genotyped for the microsatellite markers flanking these intervals. Microsatellites bounding the candidate intervals for each trait were compiled into map order based on Lexer et al. (2003a), and QTL presence was tested using interval mapping in Map Manager QTX (Manly et al. 2001). Tests were performed at 1 cm steps, and threshold values for declaring the potential presence of a QTL were determined by 1,000 permutations of the data for each trait. Three QTLs were found; two that were significant at *p* < .05 and one with marginal significance (*p* = .065). The approximate location of a QTL was established as the chromosomal region where the likelihood ratio score (LRS) exceeded the significance level.

Selection coefficients on individual QTLs contributing to adaptive traits (leaf width and stem diameter) in the *H. deserticola* environment ranged from −0.027 to −0.050 (Table 3). Although these values are somewhat lower than those reported for the *H. paradoxus* habitat, they still are considerably larger than those likely required for divergence in parapatry. Our crude estimates indicate that divergence could occur so long as the ancestral population exceeded ≈ 37 individuals, which seems plausible given that most sunflower populations number in the hundreds or thousands.

Is There Evidence of Parallel Hybrid Speciation?

Schluter (2000) argues that recurrent speciation is unlikely in the absence of parallel selective pressures resulting from adaptation to similar features in the environment. Parallel speciation is therefore viewed as "compelling evidence that divergent natural selection has ultimately brought about the evolution of reproductive isolation" (Schluter 2000, p. 378). However, there are possible exceptions to this general rule. Polyploid species, for example, frequently have multiple origins in the absence of parallel selection. Likewise, homoploid hybrid species may converge toward a similar genomic composition due to fertility selection rather than parallel natural selection (Rieseberg 2000; Rieseberg et al. 1996). However, in homoploid hybrid speciation, fertility selection does not typically confer parallel ecological changes (Rieseberg personal observations). Thus the recurrent formation of ecologically isolated homoploid hybrid species does indeed provide evidence that speciation was driven in part by divergent natural selection.

Have the homoploid hybrid species described earlier arisen multiple times? This is a difficult question to answer because patterns of molecular variation that appear most consistent with multiple hybrid speciation events almost always have alternative explanations (Gross et al. 2003). For example, the common observation that populations of the homoploid hybrid species show geographic partitioning of molecular markers can be explained by multiple hybrid speciation events or a single speciation event, followed by the sorting of ancestral variation or local introgression between the hybrid species and one of the parental species. Therefore, the evidence supporting claims of multiple origins must be considered carefully.

Argyranthemum sundingii was the first homoploid hybrid species for which multiple origins were proposed (Brochmann et al. 2000). Recurrent speciation was inferred from the presence of different chloroplast DNA types (one from each parent), in the two known populations of the species. The authors suggested that each population had been generated in situ in separate valleys from local parental populations,

although it is impossible to disprove that this pattern resulted from a single origin followed by dispersal. More recently, the researchers documented some karyotypic divergence in different populations of the hybrid species, providing further support for multiple origins (Borgen et al. 2003).

Possible multiple origins of the diploid hybrid species *P. densata* have been investigated carefully, taking advantage of the fact that chloroplast DNA is paternally inherited and mitochondrial DNA is maternally inherited in *Pinus* (Song et al. 2003; Wang et al. 2001). Patterns of variation in allozymes, chloroplast DNA, and mitochondrial DNA show that different populations of *P. desata* have very diverse genetic compositions, with varying degrees of genomic contributions from each parental species. Strikingly, each parental species has served as a maternal parent in some *P. densata* populations and a paternal parent in others (i.e., the hybrid species shows reciprocal parentage). These differences suggest that populations of the hybrid species have experienced unique evolutionary histories and most likely have independent origins.

The repeatability of diploid hybrid speciation in *H. anomalus* and *H. deserticola* was evaluated using evidence from patterns of variation in chloroplast DNA, nuclear microsatellite loci, and population cross-viability/chromosomal structure. A single origin would likely result in a single chloroplast DNA type, monophyly for the hybrid species based on microsatellites, and high interfertility among all populations. Multiple origins, on the other hand, could potentially result in multiple chloroplast DNA types, paraphyly for the hybrid species, and perhaps sharp divisions in interfertility among populations.

H. anomalus was monophyletic based on microsatellite loci, but the patterns of chloroplast DNA variation and crossability were consistent with three independent origins of the homoploid hybrid species (Schwarzbach and Rieseberg 2002). This combination of data is especially compelling because differences in chloroplast DNA type were correlated with population interfertility, a pattern that would be unlikely if variation in either trait were due to introgression with the parental species. Sorting of ancestral variation also seemed an unlikely explanation for the pattern, in that it would require that the ancestral population include chloroplast DNA from geographically distant populations of the parental populations. *H. deserticola* was paraphyletic with the parental species *H. petiolaris* based on microsatellites, and also contained chloroplast DNA types characteristic of both parental species (Gross et al. 2003). Although these patterns are suggestive of multiple origins, they were not as well supported by population interfertility as in the *H. anomalus* example.

There are now four diploid hybrid species that are thought to be the result of parallel speciation. Even if the evidence in some of these cases is due to causes other than multiple origins, it is remarkable that this outcome is supported so much more frequently than single origins, which have only been confirmed once (Welch and Rieseberg 2002). Given the limited number of homoploid hybrid species in the literature, the large proportion that appear to be multiply derived strongly implies that ecology is a frequent and significant contributor to this mode of speciation.

Conclusions

Our understanding of the ecological genetics of homoploid hybrid speciation has increased remarkably over the past five years. The rapid progress in this area reflects not only the availability of appropriate molecular tools but also the changes in prevailing views about the evolutionary forces driving hybrid speciation in response to new scientific developments. The majority of early research on homoploid hybrid speciation focused almost exclusively on the development of chromosomal isolation. Chromosomal divergence, generated via recombination of divergent parental chromosomes or de novo in hybrids, was thought to provide initial isolation between the hybrid neospecies and its progenitors, thereby facilitating further divergence. Because the process was thought to be governed primarily by drift (Grant 1958; Stebbins 1957), hybrids with the same parental species were expected to differ in genome content. Subsequent research, however, indicated that deterministic forces such as selection for fertility and viability could shape the genomes of some hybrids, apparently overcoming the effects of drift (Rieseberg et al. 1996).

As more examples of homoploid hybrid speciation were identified with molecular data, the potential importance of nonchromosomal isolating barriers became evident, especially ecological barriers (e.g., Arnold 1993; Arnold et al. 1990; Rieseberg 1991; Rieseberg 1997). These observations were incorporated into theory, which showed that homoploid hybrid species were most likely to arise under conditions of strong ecological selection and that ecological divergence was critical for persistence of the new species (Buerkle et al. 2000; McCarthy et al. 1995).

As shown in this review, empirical evidence gathered to test the role of ecological selection is consistent with theory. Essentially all of the taxa fulfill at least one of the six criteria and two species (both *Helianthus*) fulfill all six:

1. Many, if not all, homoploid hybrid species are ecologically divergent as compared to parental species, and in some cases have colonized extreme habitats.
2. Reproductive isolation is a likely outcome of the types of ecological divergence found in nature, although much more work is required to confirm this for all groups.
3. Traits found in hybrid species, presumably those facilitating the colonization of new habitats, often can be re-created via hybridization according to both greenhouse and field experiments.
4. Natural selection favoring the trait values of contemporary hybrid species' populations has been demonstrated in synthetic hybrids transplanted into the habitat of the former.
5. Selection on individual QTLs contributing to habitat isolation can be strong enough to bring about divergence even in the presence of gene flow with parental populations.

6. Molecular evidence suggests parallel homoploid hybrid speciation is the rule rather than the exception.

Finally, comparative mapping studies in *Helianthus* have shown that the current genomic composition of the homoploid hybrid species corresponds very closely to what is predicted based on the phenotypes of those species and the QTLs mapped in the parental species. Thus, habitat mediated selection acting on transgressive hybrids allowed adaptation to the extreme habitats where the ancient hybrid species are now found. Although this comparative mapping approach may not be feasible in all systems, it provides the strongest evidence that natural selection acting on variation created by hybridization created the phenotypes we now see in nature.

It is important to note that although the experimental work to date has been a useful first step toward understanding the ecological genetics of homoploid hybrid speciation, research is complicated by the fact that reproductive isolation in many groups may be based on both ecological (habitat divergence) and nonecological (chromosomal differences) factors. For example, Kirkpatrick and Barton (1997) argue that habitat colonization is inhibited by gene flow from the center of a species' range rather than limited genetic variance. If they are correct, then it might be that ecological divergence in hybrid lineages is made possible by chromosomal isolation rather than new hybrid gene combinations. Information from systems where reproductive isolation is purely ecological would be especially useful in this regard. Of course, the results of such work would be interesting not only in reference to homoploid hybrid speciation but also because of implications for the closely related phenomena of sympatric and ecological speciation (Schluter 2001; Via 2001).

Acknowledgments

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