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Abstract.—If two previously isolated taxa mutually assimilate through hybridization and subsequent biparental introgression, and if their introgressed descendants have the same or higher fitness than their parents, then gene flow should result in the local extinction of parental taxa via replacement by hybrid derivatives. These dramatic events may occur rapidly, even in a few generations. Given the speed at which such extinction by hybridization may occur, it may be difficult to identify that the process has occurred. Thus, documented instances of extinction by hybridization are rare, and especially so for cases in which both parents are replaced by the hybrid lineage. Here we report morphological and allozyme evidence for the local extinction of two Raphanus species in California via replacement by their hybrid-derived descendants. The results from a greenhouse experiment demonstrate that California wild radishes have a specific combination of traits from their progenitors, and comparison of our results to that of an earlier report indicate that pure parental types are no longer present in the wild. Our results also show the hybrid-derived lineage has transgressive fruit weight compared to its parents. Allozyme analysis demonstrates that California wild radishes are derived from hybridization between the putative parental species. However, that analysis also demonstrates that California wild radish has now become an evolutionary entity separate from both of its parents. We suggest that the aggressive colonizing behavior of the hybrid-derived lineage probably results from a novel combination of parental traits, rather than genetic variability of the population per se.

Key words.—Allozymes, genetic assimilation, hybridization, introgression, local extinction, Raphanus raphanistrum, Raphanus sativus.

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The extinction of plants and animals through hybridization is receiving increasing attention as a legitimate conservation problem (Rieseberg and Gerber 1995; Levin et al. 1996; Rhymer and Simberloff 1999; Huxel 1999; Ferdy and Austerlitz 2002; Rhymer and Simberloff 2006). The extinction of two previously isolated taxa mutually assimilate through hybridization and subsequent biparental introgression, and if their introgressed descendants have the same or higher fitness than their parents, then gene flow should result in the local extinction of parental taxa via replacement by hybrid derivatives. These dramatic events may occur rapidly, even in a few generations. Given the speed at which such extinction by hybridization may occur, it may be difficult to identify that the process has occurred. Thus, documented instances of extinction by hybridization are rare, and especially so for cases in which both parents are replaced by the hybrid lineage. Here we report morphological and allozyme evidence for the local extinction of two Raphanus species in California via replacement by their hybrid-derived descendants. The results from a greenhouse experiment demonstrate that California wild radishes have a specific combination of traits from their progenitors, and comparison of our results to that of an earlier report indicate that pure parental types are no longer present in the wild. Our results also show the hybrid-derived lineage has transgressive fruit weight compared to its parents. Allozyme analysis demonstrates that California wild radishes are derived from hybridization between the putative parental species. However, that analysis also demonstrates that California wild radish has now become an evolutionary entity separate from both of its parents. We suggest that the aggressive colonizing behavior of the hybrid-derived lineage probably results from a novel combination of parental traits, rather than genetic variability of the population per se.
The scenario is plausible: examples of hybrid fitness equal and superior to that of their parents are well known and increasing (Wilson and Brown 1953; Brochmann 1984; Wynn 1986; Echelle and Connor 1989; Arnold and Hodges 1995; Rieseberg et al. 1999). One example is the rusty crayfish, *Orconectes rusticus*, which was introduced to northern Wisconsin and Michigan lakes and streams around 1979 (Perry et al. 2001). There, it hybridizes with the native *O. propinquus*. Fecundity of hybrids and early hybrid survivorship do not differ significantly from nonhybrids. Moreover, adults of mixed ancestry were found superior to both *O. propinquus* and *O. propinquus* when competing for a limiting food resource. Perry et al. (2001) concluded that hybridization and introgression were accelerating the elimination of genetically pure *O. propinquus*.

Most empirical studies focus on unilateral introgression; that is, hybridization as an extinction threat to only one of the hybridizing parents. Unilateral introgression is probably more frequent than bilateral introgression because the probability of asymmetric gene flow is more likely than symmetric gene flow (Ellstrand and Elam 1993). In particular, if selection is absent, for a locally abundant and rare pair of sympatric congeners, we would expect gene flow from the common species to dominate the local mating dynamics of the hybrid swarm (Wolf et al. 2001).

Only a few reports describe bilateral introgression so substantially that hybrid lineages have genetically overwhelmed both parental species (Levin et al. 1996). For example, in several locations in the American Midwest, the amaranths, *Amaranthus* and *A. tuberculatus*, have hybridized so extensively that hybrid derivatives have apparently completely replaced their parents (Sauer 1957). Likewise, the hybrid zone between the striped species, *Piriqueta caroliniana* and *P. viridis*, in central Florida is extensive and spreading (Martin and Cruzan 1999).

Even more extensive hybridization occurs for cultivated radish, *Raphanus sativus*, and jointed charlock, *R. raphanistrum*, in California. *Raphanus sativus* has white, pink, or purple flowers and a swollen taproot. Its fruits are smooth, spongy, and corky, easily crushed by hand. Jointed charlock has white or yellow flowers, a slim taproot with frequent branching, and fruits with constrictions between the seeds and is “not crushable, except between the seeds, by hand pressure” (Panetsos and Baker 1967, p. 246). These two species are native to Europe where they occasionally hybridize but maintain their integrity as separate species (Clapham et al. 1987; Stace 1991). Both species were introduced into California well over a century ago (Bolander 1870; Brewer et al. 1876; Panetsos and Baker 1967) and have become naturalized in California (Hickman 1993). Apparent hybrids have been known from California for almost a century (Frost 1923). Four decades ago, Panetsos and Baker (1967) conducted a detailed morphological and cytotenic investigation of central California *Raphanus* populations from the Sierra Nevada foothills to the Pacific coast. They found pure *R. sativus* populations near the coast and pure *R. raphanistrum* populations only in inland areas distant from the coast. Occupying the region between the pure populations, they found several populations composed of individuals with combinations of traits of the two *Raphanus* species, which they called “‘wild’ *R. sativus*. In artificial crosses between *R. raphanistrum* and *R. sativus*, they also noticed the absence of a prezygotic mating barrier and the presence of a single reciprocal translocation difference between the two *Raphanus* species. Based on these data, Panetsos and Baker (1967) suggested that wild *R. sativus* populations evolved from hybrids between *R. sativus* and *R. raphanistrum*. California’s wild radishes have also become an important weed in the state (Ball et al. 2000).

California *Raphanus* (hereafter referred to as “California wild radish” or “wild radish”) populations have continued to evolve. Since 1983, as part of numerous wild radish studies (Ellstrand and Marshall 1985; Nason and Ellstrand 1995), we visited more than 50 wild radish populations from Cannon Beach, Oregon, through California and south to Santo Tomas, Northern Baja California, Mexico, including both coastal sites and inland valleys, a transect of over 2000 km. At least 20 plants were inspected in each population. At all these sites, almost all plants combined features of both cultivated radish and jointed charlock. Plants typically had unswollen roots and either spongy fruits with one or a few weak constrictions or hard fruits without constrictions. Thus, the vast majority of the plants could not be assigned to either parental species.

Apparently, since Panetsos and Baker’s (1967) study, the remaining pure *R. sativus* and *R. raphanistrum* populations have been completely replaced by wild radish populations.

The foregoing observations of wild radish populations were based on morphology. However, morphology alone is insufficient to judge the extent or pattern of hybridization and introgression that has occurred (Gottlieb 1972). For example, it is impossible to judge by morphology whether wild radish in California represents a genetically assimilated lineage or, for example, individuals of largely genetically pure *R. raphanistrum* or *R. sativus* with a few introgressed loci derived by repeated backcrossing. If the two species have completely assimilated, we expect that the populations should have proportions of allele frequencies of the progenitors that vary over loci; if they are the result of repeated backcrossing to one taxon, then allele frequencies should be much more uniformly close to that taxon. Likewise, if the two species have completely integrated across their range, we expect individual loci in all populations to be in Hardy-Weinberg (HW) equilibrium; if introgression has been arrested by residual isolating barriers or by selection against the hybrids, we expect significant levels of heterozygote deficiency at a number of loci. Furthermore, if introgressed lineages are selected to possess a specific combination of parental traits, California wild radish populations should have specific combinations of alleles and of genetically based morphological characters. In this context, the specific objectives of this study were to use genetically based polymorphic morphological traits and allozyme loci to (1) quantitatively determine the current morphological status of numerous California wild radish populations relative to their putative progenitors, and (2) determine the genetic constitution of individuals in California wild radish populations to test whether they have evolved as a result of bilateral genetic assimilation between *R. sativus* and *R. raphanistrum*, thereby replacing both progenitors.
**Fig. 1.** Sampling locations of California wild radish populations.

**Materials and Methods**

**Greenhouse Experiment**

A greenhouse experiment was conducted at the University of California Agricultural Experiment Station at Riverside, California. The objective of this experiment was to compare eight key morphological traits of wild radish with the potential parental *Raphanus* species. The experiment included 24 wild radish populations collected from both coastal and inland sites of California (Fig. 1; Appendix). Because we were not able to locate any morphologically pure *R. raphanistrum* populations in California, the two *R. raphanistrum* populations included in the experiment were collected outside of California, one from Michigan and the other from the Botanical Garden of Roskilde University, Roskilde, Denmark (Appendix). At present, about 20–30 cultivars are sold in California. These cultivars generally come in red and white root colors, although a black root type radish is also grown in a few locations in California. Historically and currently (Randolph 1793; George and Evans 1980), red and white radish cultivars have been most popular in Europe and California. Accordingly, we chose four cultivars—two red and two white—that have been in cultivation for decades in California (Appendix) for our greenhouse experiment.

From each of the 30 (24 wild radishes, two *R. raphanistrum*, four cultivar radishes) above-mentioned populations, one seed each from 10 randomly selected individuals was planted in 1-gal. pots in a greenhouse. *Raphanus* species are self-incompatible and insect pollinated (Kay 1976; Stanton 1987; Karron et al. 1990). Natural intrapopulation fruit set was accomplished by removing the plants by population from the greenhouse to allow pollinators (mainly honeybees) to have access to the flowers over a period of several hours. Subsequently, all unopened buds and extra branches were stripped off, the branches tied to a stake, and the plants were returned to the greenhouse until the fruits matured.

The following measurements were recorded: bolting date, flowering date, root length, root width, fruit diameter, fruit shape (length-to-diameter ratio), and fruit weight. The root colors were not scored or analyzed as other morphological traits. The root length was measured as length of the whole main root from the upper end of the hypocotyls to the point at which the main root either branches or ends. The root width was measured as diameter of the hypocotyl at the widest point. Fruit diameter was measured at its widest point. Fruit shape was expressed as a ratio of fruit length (measured from base to tip) to diameter. All measurements of fruit morphology (including fruit weight) were recorded as an average of three randomly selected fruits from each individual. Flower color was scored as white, purple, pink, bronze, or yellow and was not included as a variable in statistical analyses. Morphological data were tested for univariate normality using normal probability plots. Non-normal data (bolting date, flowering date, root length, root width) were log transformed and all quantitative morphological traits were tested for significant trait differences among three *Raphanus* taxa using a linear mixed model analysis of variance (ANOVA) procedure treating *Raphanus* taxa as a fixed effect factor and populations within each taxon as a random effect factor.

The differences among all three *Raphanus* taxa according to means of seven morphological traits were tested using general linear model (GLM) multivariate procedure (MANOVA). Morphological data were tested for multivariate normality and equality of variance-covariance matrices using Box’s M test and spread-versus-level plots. Pairwise multiple comparisons of the estimated marginal means (the group means that were estimated from the fitted model) of morphological traits between taxa were performed using the Sidak test for both ANOVA and MANOVA procedures. Discriminant function analysis (DFA) was applied to the same set of morphological data, described above, to identify the set of variables that discriminate three *Raphanus* taxa, to determine how well the three taxa can be separated, and to test whether the observed samples were correctly classified to dependent groups as predicted. A stepwise DFA was used to eliminate variables with very little or no contributions to discriminant functions. The Wilk’s method, with the probability of F criterion for the entry and removal of variables into equations, was employed to identify the predictor variables for the stepwise DFA. For MANOVA and DFA, all seven morphological traits were log-transformed to reduce the inequality of variance-covariance matrices. Initially a set of discriminate functions was generated using 70% of original grouped cases involving all three *Raphanus* taxa and all seven morphological traits. Cross-validation of the discriminant functions was done by estimating the percentage of correct classification of selected and unselected original grouped cases. Discriminant functions were tested for their significance.
using Wilk’s lambda. All statistical analyses were performed using SPSS (SPSS 2002).

Allozyme Analysis

Allozyme frequencies were obtained for three wild *R. raphanistrum* populations, 10 California wild radish populations, and four *R. sativus* cultivars (Table 1). Except for three California wild radish populations (Marin Co., Mendocino Co., Sacramento Co., Table 1), the remaining seven populations were collected from the same locations used in the common garden experiment. Likewise, all four cultivars were common for both greenhouse and allozyme experiments. All three *R. raphanistrum* populations for allozyme analysis were selected from Europe. We had three reasons for choosing European jointed charlocks instead of those from the United States for population genetic analysis. First, a few plants from the Michigan *R. raphanistrum* populations that were originally used in the greenhouse experiment showed segregation for morphological traits associated with the cultivated species, possibly as a result of historical gene flow from cultivated types. Second, pure natural populations of *R. raphanistrum* populations that were originally used in the greenhouse experiment showed congruence with the evolutionary history of California wild radish. We did not include the Denmark population used in the greenhouse experiment for the allozyme analysis due to lack of sufficient sample size.

We analyzed 20–80 plants from each source. Zymograms were obtained by the electrophoresis of fresh plant material on a 10% starch gel and subsequent staining. The following 10 allozyme loci were scored: Phosphoglucomutase-1 (*Pgm-1*, E.C. 5.4.2.2), Phosphoglucomutase-2 (*Pgm-2*), Phosphoglucomutase-3 (*Pgm-3*), Isocitrate dehydrogenase (*Idh*, EC 1.1.1.42), Phosphoglucoisomerase (*PgI*, E.C. 5.3.1.9), Aconitase (*Aco*, E.C. 4.2.1.3), 6-Phosphogluconate dehydrogenase (*Pgd*, E.C. 1.1.1.44), Triose-phosphate isomerase (*Tpi*, E.C. 5.3.1.1), Leucine aminopeptidase (*Lap*, E.C. 3.4.11.1), Acid phosphatase (*Acp*, E.C. 3.1.3.2). Details for radish allozyme analysis from extraction through staining as well as allozyme inheritance patterns are reported elsewhere (Ellstrand and Devlin 1989). None of these 10 allozyme loci exhibits any genetic linkage (Conner et al. 1997) in *R. raphanistrum* populations, but in wild radish linkage was detected between *Pgm-2*, *Aco*, *Acp*, and *Lap* (Ellstrand and Devlin 1989).

Allozyme frequency data and genetic diversity statistics (Nei 1987) were analyzed using POPGEN version 1.32 (Yeh and Boyle 1997). Observed heterozygosity (*H*o) was estimated as the proportion of observed heterozygotes for a given locus averaged over all 10 loci within a population for the population level estimate and over all 10 loci of all populations within a taxon for the species-level estimate. Expected heterozygosity (*H*e) was estimated as the proportion of expected heterozygotes under random mating (Nei 1973) averaged over all 10 loci within a population for the population level estimate and over all 10 loci of all populations within a taxon for the species level estimate. Polymorphic loci were calculated as the number of loci that were polymorphic irrespective of allele frequencies. Hardy-Weinberg equilibrium for each locus was calculated based on expected genotypic frequencies under random mating using the algorithm by Levin (1949); the deviations from HW equilibrium were tested for their significance at the 5% level of probability using the chi-square test. The adjusted *P*-values for multiple comparisons were determined following the sequential Bonferroni method (Rice 1989). *F*ST values (Nei 1987) were calculated for each species.

We examined the extent and nature of introgression in wild radish populations using a recently developed statistical genetic clustering algorithm called ‘*structure*’ (Pritchard et al. 2000). This computer program is a model-based clustering method for inferring population structure using genotype data. The clustering method assumes a model in which there are *K* populations (where *K* may be unknown), each of which is characterized by a set of distinct allele frequencies at each locus. Individuals in the sample are probabilistically (Bayesian probability) assigned to populations, or jointly to two or more populations—if their genotypes indicate that they are admixed—following a Markov chain Monte Carlo scheme.
The program depicts the nature and extent of genetic admixture at both population and individual levels, thus facilitating identification of even the cryptic introgression events between closely related taxa. Application of the method includes demonstrating the presence of population structure, assigning individuals to populations, and identification of introgression events as subtle as those between closely related taxa. The analysis was carried out assuming that individuals in the populations may have mixed ancestry and that allele frequencies in different populations are reasonably different from each other. The analysis was carried out without specifying the sampling locations of the populations and assuming that loci are unlinked and can freely recombine. The simulation had a burn-in period of 10,000 iterations and run length of 1,000,000 iterations. The number of clusters (K) was determined both from an earlier report of hybrid origin for California wild radishes (Panetsos and Baker 1967) and from the value of K that maximized the estimated model log-likelihood.

**Results**

The results of our greenhouse experiment (Table 2) showed that the morphological relationship of California wild radishes to their parents varies with the specific trait measured. California wild radishes were intermediate with respect to bolting date, flowering date, root length, and root width and were significantly different from both parents. Wild radishes had significantly larger fruit diameter and smaller fruit shape than *R. raphanistrum*; but these two characters were not significantly different from *R. sativus*. Wild radishes also transgress both parents for one trait: significantly greater fruit weight (Table 2). Flower colors of individual wild radish populations generally included white, purple, pink, bronze, and yellow, segregating the colors of both parents, but were occasionally pure white or yellow.

The MANOVA test revealed that all three *Raphanus* taxa differed significantly in overall plant morphology (*F* = 39.79, *P* < 0.000, with 14 and 562 degrees of freedom, Pillai’s trace test). Two different sets of combinations among five morphological traits—bolting date, flowering date, root width, root length, and pod diameter—effectively separated all three *Raphanus* taxa in a multidimensional variable space (Table 3A; Fig. 2). The first two canonical discriminant functions accounted for 100% of variance in the data (*DF* 1 = 90% and *DF* 2 = 10%). Although high scores on the *DF* 1 resulted from the high values of root width, flowering date, and root length combined with the low values of bolting date and fruit diameter, the high scores on the *DF* 2 resulted from the high values of fruit diameter and bolting date combined with the low values of root width, flowering date, and root length (Table 3A). Furthermore, the *DF* 1 was relatively more effective in separating *R. sativus* individuals from *R. raphanistrum* and wild radish individuals combined, whereas the *DF* 2 further separated the wild radish group from the *R. raphanistrum* group (Fig. 2; Table 3B), suggesting a significant difference between *DF* 1 and *DF* 2 in their ability to distinguish among three *Raphanus* taxa (*DF* 1 through *DF* 2. Wilk’s λ = 0.21, χ² = 315.22, *P* < 0.000, df = 10; *DF* 2, Wilk’s λ = 0.77, χ² = 52.96, *P* < 0.000, df = 4). The two discriminant functions performed equally well for *R. raphanistrum* and *R. sativus* groups by correctly classifying 100% of the cases to their original groups. Although a large percentage (85% of the cases) of wild radish individuals were correctly classified, a few cases were misclassified as *R. raphanistrum* (13%) or *R. sativus* (2%)—a fairly common phenomenon observed among introgressed individuals.

The allozyme frequencies for the three *Raphanus* groups are reported in Table 4. The similarity of the wild radish populations to their parents varies depending on the locus. For four loci (*Pgm-1, Pgm-3, Tpi, Acp*), the allele frequencies of the wild radishes were similar to those of jointed charlock.

### Table 2. Pairwise comparisons of estimated marginal means of morphological characters based on the linear mixed model procedure.

<table>
<thead>
<tr>
<th>Traits</th>
<th>R. raphanistrum n² = 20</th>
<th>Wild radish n = 229</th>
<th>R. sativus n = 40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bolting date</td>
<td>22.39 a (19.95–25.70)</td>
<td>31.62 a (29.51–33.11)</td>
<td>48.98 a (43.65–54.95)</td>
</tr>
<tr>
<td>Flowering date</td>
<td>34.67 a (30.90–38.90)</td>
<td>43.65 b (41.69–45.71)</td>
<td>69.18 a (66.07–81.28)</td>
</tr>
<tr>
<td>Flower color</td>
<td>yellow</td>
<td>white, purple, pink, bronze, yellow</td>
<td>purple and white</td>
</tr>
<tr>
<td>Root length (cm)</td>
<td>11.22 a (9.95–13.18)</td>
<td>14.45 a (13.18–15.49)</td>
<td>17.38 a (14.79–19.95)</td>
</tr>
<tr>
<td>Root width (cm)</td>
<td>0.87 a (0.72-1.06)</td>
<td>1.74 a (1.58–1.95)</td>
<td>5.12 b (4.27–6.17)</td>
</tr>
<tr>
<td>Fruit diameter (mm)</td>
<td>1.41 a (0.37–2.46)</td>
<td>6.24 b (5.71–6.77)</td>
<td>8.02 b (6.49–9.56)</td>
</tr>
<tr>
<td>Fruit shape</td>
<td>15.09 a (13.48–16.70)</td>
<td>8.90 a (8.14–9.65)</td>
<td>6.10 b (5.10–7.10)</td>
</tr>
<tr>
<td>Fruit weight (g)</td>
<td>0.11 a (0.03–0.20)</td>
<td>0.33 a (0.29–0.36)</td>
<td>0.08 a (0.005–0.162)</td>
</tr>
</tbody>
</table>

1 Bolting date, flowering date, root length, and root width are geometric means of back-transformed values, and fruit diameter, shape and weight are arithmetic means.

2 Sample size.

3 Mean values with different superscripts are significantly different at *P* < 0.05.

4 Confidence interval.

### Table 3. Standardized canonical discriminant function coefficients of five morphological characters (A), and unstandardized canonical discriminant functions evaluated at group means (centroids) (B) of three *Raphanus* taxa.

<table>
<thead>
<tr>
<th>A. Traits</th>
<th>Function 1</th>
<th>Function 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root width</td>
<td>0.91</td>
<td>-0.46</td>
</tr>
<tr>
<td>Flowering date</td>
<td>0.48</td>
<td>-0.24</td>
</tr>
<tr>
<td>Root length</td>
<td>0.21</td>
<td>-0.18</td>
</tr>
<tr>
<td>Bolting date</td>
<td>-0.36</td>
<td>0.48</td>
</tr>
<tr>
<td>Fruit diameter</td>
<td>-0.07</td>
<td>1.07</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Taxa</th>
<th>Function 1</th>
<th>Function 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. raphanistrum</td>
<td>-1.66</td>
<td>-1.94</td>
</tr>
<tr>
<td>Wild radish</td>
<td>-0.54</td>
<td>0.20</td>
</tr>
<tr>
<td>R. sativus</td>
<td>4.02</td>
<td>-0.24</td>
</tr>
</tbody>
</table>
For three loci (Idh, Aco, Lap), the frequencies were more or less intermediate to the two parents. For three loci (Pgm-2, Pgi, Pgd), frequencies were radically different from both parents.

The majority of the polymorphic loci were in HW equilibrium both in parental and wild radish populations (Table 5). Most significant for our study, of the 10 wild radish populations, five populations had all of the polymorphic loci in HW equilibrium. In the remaining five populations, nine of the 10 polymorphic loci were in HW equilibrium.

Overall genetic diversity estimates (Table 5, genetic diversity, $H_e$, at taxon level) were higher in wild radishes than the parental taxa. The same was true for most genetic diversity estimates at the population level. The between-population genetic diversity ($F_{ST}$) in wild radish was substantially lower than the parental populations sampled.

Figure 3 displays the results from the structure analysis. The width of each numbered and color-coded panel represents the sample size of each population, ranging anywhere from 18 to 80 individuals. The *Raphanus* populations are assigned the same numbers listed in Table 1: numbers 1–3 are European jointed charlock populations, 4–13 are California wild radish populations, and 14–17 are the four radish cultivars populations. Assignment of all individuals to two clusters ($K = 2$) reveals relative genotypic purity of *R. raphanistrum* (red) and *R. sativus* (green). The average proportion of membership of three *R. raphanistrum* populations in the red cluster is 0.87 and that of four *R. sativus* populations is 0.93 in the green cluster. At this level of clustering, all the individuals from California wild radish populations reveal some level of genetic admixture from the parental taxa (average membership proportions of 0.54 and 0.46 in red and green clusters, respectively). These results are in accord with the hypothesis that wild radish populations originated through biparental introgression.

Discussion

The results of both our greenhouse experiment and our descriptive study with genetic markers support Panetsos and Baker’s (1967) hypothesis of a hybrid origin of California’s wild radish populations. However, they also show that the situation has been dynamic. In contrast to Panetsos and Baker’s (1967) findings of localized populations of hybrid derivatives flanked at the regional scale by populations of more or less genetically pure parents, we found no pure parental types in the wild in California. Instead, throughout California, we found *Raphanus* plants with a single widespread and more or less uniform morphology that, taken as a whole, did not match either *R. sativus* or *R. raphanistrum* (Table 2, Fig. 2).

Panetsos and Baker (1967) reported two pure populations of *R. raphanistrum* in the interior locations of the Central Valley, California, based on their fruit diameter and flower color observations. The two populations had an average fruit diameter of less than 5.1 mm and had both yellow (85–93%) and white (7–15%) flowers. On the contrary, all their presumed wild radish populations had a mosaic of flower colors (white, pink, purple, yellow, or bronze) and had an average fruit diameter falling within the range of 5.1 mm to 8.1 mm. The one cultivar used in their study had pink flowers with a fruit diameter averaging more than 8.1 mm. Today both agriculture and real estate development have replaced the two pure *R. raphanistrum* populations mentioned in Panetsos and Baker’s (1967) study. Nevertheless, other nearby populations (Fig. 1, Appendix) used in our investigation did not possess the two distinct traits—predominantly yellow flowers and fruit diameter of less than 5.1 mm—observed by Panetsos and Baker (1967). All 24 wild radish populations in our study had an average fruit diameter of 6.24 mm and had high proportions of flower colors other than yellow (Table 2). Likewise, their low $F_{ST}$ suggests relative genetic uniformity for
that a hybrid lineage has genetically absorbed and/or com-
gene flow, or both. The most parsimonious interpretation is
allozyme loci due to a common phylogenetic heritage, high
Acp
A 0.03 0.03 0.82
Lap
Tpi
A 0.02 0.25 0.00
Pgd
Aco
A 0.25 0.60 0.11
B 0.56 0.40 0.89
C 0.19 0.00 0.00
Pgm-2
A 0.03 0.14 0.49
B 0.97 0.48 0.28
C 0.13 0.38 0.23
Pgm-3
A 0.08 0.01 0.01
B 0.09 0.09 0.20
C 0.82 0.84 0.80
D 0.01 0.06 0.00
Idh
A 0.16 0.03 0.00
B 0.32 0.66 0.76
C 0.52 0.29 0.24
D 0.00 0.02 0.00
Pgi
A 0.38 0.03 0.00
B 0.06 0.33 0.23
C 0.15 0.24 0.64
D 0.16 0.20 0.01
E 0.13 0.05 0.00
F 0.12 0.14 0.12
Aco
A 0.25 0.60 0.11
B 0.56 0.40 0.89
C 0.19 0.00 0.00
Pgd
A 0.02 0.25 0.02
B 0.16 0.02 0.00
C 0.82 0.73 0.98
Tpi
A 0.75 0.78 1.00
B 0.25 0.22 0.00
Lap
A 0.04 0.01 0.00
B 0.30 0.15 0.01
C 0.25 0.49 0.49
D 0.04 0.07 0.01
E 0.30 0.15 0.00
F 0.07 0.13 0.49
Acp
A 0.03 0.03 0.82
B 0.96 0.72 0.18
C 0.01 0.25 0.00
R. raphanistrum
(n = 74)
Wild radish
(n = 368)
R. sativus
(n = 80)

<table>
<thead>
<tr>
<th>Locus</th>
<th>Alleles</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pgm-1</td>
<td>A 0.00 0.02 0.00</td>
<td>B 0.00 0.22 0.73</td>
</tr>
<tr>
<td></td>
<td>C 0.68 0.57 0.04</td>
<td>D 0.32 0.19 0.23</td>
</tr>
<tr>
<td>Pgm-2</td>
<td>A 0.03 0.14 0.49</td>
<td>B 0.97 0.48 0.28</td>
</tr>
<tr>
<td></td>
<td>C 0.13 0.38 0.23</td>
<td></td>
</tr>
<tr>
<td>Pgm-3</td>
<td>A 0.08 0.01 0.01</td>
<td>B 0.09 0.09 0.20</td>
</tr>
<tr>
<td></td>
<td>C 0.82 0.84 0.80</td>
<td>D 0.01 0.06 0.00</td>
</tr>
<tr>
<td>Idh</td>
<td>A 0.16 0.03 0.00</td>
<td>B 0.32 0.66 0.76</td>
</tr>
<tr>
<td></td>
<td>C 0.52 0.29 0.24</td>
<td>D 0.00 0.02 0.00</td>
</tr>
<tr>
<td>Pgi</td>
<td>A 0.38 0.03 0.00</td>
<td>B 0.06 0.33 0.23</td>
</tr>
<tr>
<td></td>
<td>C 0.15 0.24 0.64</td>
<td>D 0.16 0.20 0.01</td>
</tr>
<tr>
<td></td>
<td>E 0.13 0.05 0.00</td>
<td>F 0.12 0.14 0.12</td>
</tr>
<tr>
<td>Aco</td>
<td>A 0.25 0.60 0.11</td>
<td>B 0.56 0.40 0.89</td>
</tr>
<tr>
<td></td>
<td>C 0.19 0.00 0.00</td>
<td></td>
</tr>
<tr>
<td>Pgd</td>
<td>A 0.02 0.25 0.02</td>
<td>B 0.16 0.02 0.00</td>
</tr>
<tr>
<td></td>
<td>C 0.82 0.73 0.98</td>
<td></td>
</tr>
<tr>
<td>Tpi</td>
<td>A 0.75 0.78 1.00</td>
<td>B 0.25 0.22 0.00</td>
</tr>
<tr>
<td>Lap</td>
<td>A 0.04 0.01 0.00</td>
<td>B 0.30 0.15 0.01</td>
</tr>
<tr>
<td></td>
<td>C 0.25 0.49 0.49</td>
<td>D 0.04 0.07 0.01</td>
</tr>
<tr>
<td></td>
<td>E 0.30 0.15 0.00</td>
<td>F 0.07 0.13 0.49</td>
</tr>
<tr>
<td>Acp</td>
<td>A 0.03 0.03 0.82</td>
<td>B 0.96 0.72 0.18</td>
</tr>
<tr>
<td></td>
<td>C 0.01 0.25 0.00</td>
<td></td>
</tr>
</tbody>
</table>

allozyme loci due to a common phylogenetic heritage, high
gene flow, or both. The most parsimonious interpretation is
that a hybrid lineage has genetically absorbed and/or com-
petitively displaced both parental types.

Given the high levels of compatibility among the three
types (Panetsos and Baker 1967; Klinger et al. 1991; Snow
et al. 2001), it is clear that genetic absorption should proceed
quite easily. But given the limited (Table 2), but distinct (Fig.
3), variation of morphology of the wild radish plants we
observed, it is clear that these populations are not simple
hybrid swarms. Parental types are not segregating in the wild.
Natural selection must have played a role in the evolution
and spread of California’s wild radish—perhaps involving
the competitive exclusion of the parental types in addition
to their assimilation.

One reason that we successfully identified hybridization
as the cause of extinction of the two parental species is that
the whole system is created between two naturalized aliens,
whose history of introduction is fairly well documented. It
would have been much more difficult, if not impossible, to
reconstruct the evolutionary events without historical infor-
mation. As anticipated by theoretical work (Huxel 1999; Wolf
et al. 2001), our data demonstrate that approximately 100
years (less than 100 generations for an annual with a sub-
stantial seed bank) is more than sufficient for extinction by
hybridization to occur.

Our allozyme data and structure analysis (Fig. 3) also sup-
port this interpretation. The populations are largely in HW
equilibrium, supporting the idea that they are not harboring
cross-incompatible subpopulations, and that the phenomenon
is not an artifact of a few isolated wild radish populations;
rather, it is a general feature of the majority of wild radish
populations in California (Table 5). Furthermore, despite the
fact that California wild radishes are much more genetically
diverse than their parents, the $F_{ST}$ value for California wild
radish populations is low enough to suggest a very close
genetic relationship among them (Table 5). In addition, struc-
ture analysis using the allozyme data for assignment to two
clusters reveals the admixed nature of the contemporary Cal-
ifornia wild radish individuals. Furthermore, the HW equi-
librium at loci in mixed populations clearly shows that Cal-
ifornia wild radish individuals comprise a separate evolu-
tionary unit from their parents (Figs. 2, 3). Interestingly, the
allozyme data combined with those discussed above regard-
ing uniformity of morphology suggest that genetic diversity
per se does not currently contribute to the success of Cal-
ifornia wild radish.

California wild radish’s evolutionary success most likely
resulted from the evolution of a unique combination of traits
relative to its progenitors (Fig. 3, Table 2; Pillai’s trace test,
$P < 0.000$ in MANOVA). We noticed that wild radish pop-
ulations have unswollen roots and early flowering similar to
R. raphanistrum. Although wild radishes and cultivars pos-
sess very similar fruit size, wild radish fruits are harder to
break open compared to the soft cultivar pods (S. G. Hegde,
pers. obs.). Another notable difference between wild radish
and parents is the transgressive pod weight in wild radish
populations (Table 2). Cultivars are selected for domesticated
traits—namely, delayed flowering to facilitate root enlarge-
ment, intact fruits to enhance seed harvest, and soft fruits to
assist easy seed extraction—that are disadvantageous in the
wild. For example, the swollen root is sensitive to disease
and mechanical injuries, and its limited absorbing capacity
restricts cultivars to rich and moist soil. In addition, the soft,
intact fruits are an easy prey for birds (Frost 1923; Panetsos
and Baker 1967; Klinger et al. 1991; S. G. Hegde, pers. obs.)
and are not efficient seed dispersers. Not unexpectedly, none
of those traits was present in California wild radish. The
weedy parent, R. raphanistrum, has slender and well-
branched roots, takes a brief time from germination to flow-
ering, and possesses very hard fruits that break at joints.
These properties make jointed charlock a successful weed of
dry areas (Panetsos and Baker 1967). The spongy fruits of
California wild radish appear to have evolved as an adap-
tation to protect seeds from predation by seed-eating birds.
For example, California’s ubiquitous house finches are able
to consume seeds from the narrow stylar tips of the fruits,
but not those seeds situated in the broader stem end. Also,
when the fruits desicce, they often leave a single seed still
attached to the plant, a built-in dispersal polymorphism (D.
Table 5. Genetic diversity statistics. \( H_o \), observed heterozygosity at population or species level; \( H_e \), expected heterozygosity or genetic diversity at population or species level; \( F_{ST} \), genetic variation between populations or species.

<table>
<thead>
<tr>
<th>Population level estimates (averages of 10 loci)</th>
<th>Taxon level estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n )</td>
<td>( H_o )</td>
</tr>
<tr>
<td>R. raphanistrum</td>
<td></td>
</tr>
<tr>
<td>1. Germany</td>
<td>26</td>
</tr>
<tr>
<td>2. Belgium</td>
<td>27</td>
</tr>
<tr>
<td>3. Italy</td>
<td>20</td>
</tr>
<tr>
<td>Wild radish</td>
<td></td>
</tr>
<tr>
<td>4. Mendocino</td>
<td>20</td>
</tr>
<tr>
<td>5. Marin</td>
<td>15</td>
</tr>
<tr>
<td>6. Santa Barbara</td>
<td>18</td>
</tr>
<tr>
<td>7. San Diego</td>
<td>20</td>
</tr>
<tr>
<td>8. Colusa</td>
<td>30</td>
</tr>
<tr>
<td>9. Sutter</td>
<td>31</td>
</tr>
<tr>
<td>10. Sacramento</td>
<td>44</td>
</tr>
<tr>
<td>11. Riverside-1</td>
<td>80</td>
</tr>
<tr>
<td>12. Riverside-2</td>
<td>45</td>
</tr>
<tr>
<td>13. Riverside-3</td>
<td>43</td>
</tr>
<tr>
<td>R. sativus</td>
<td></td>
</tr>
<tr>
<td>14. Cherry Belle-1</td>
<td>30</td>
</tr>
<tr>
<td>15. Cherry Belle-2</td>
<td>20</td>
</tr>
<tr>
<td>16. Giant White Globe-1</td>
<td>30</td>
</tr>
<tr>
<td>17. Giant White Globe-2</td>
<td>20</td>
</tr>
</tbody>
</table>

Radish appears to have been selected for a combination of fitness traits different from those of its parents (Table 2, Fig. 2), and perhaps better able to cope with the diverse ecosystems of California.

The number of studies describing the mixing of two parental taxa into a stabilized hybrid lineage is slowly increasing (Wynn 1986; Echelle and Connor 1989; Martin and Cruzan 1999; Salzburger et al. 2002; Taylor 2004; Taylor et al. 2006). For instance, Echelle and Connor (1989) have reported extensive and rapid genetic introgression between endemic and introduced pupfish species in part of the Pecos River in Texas. Within four years, the panmictic admixture of these two pupfishes occupied approximately 430 river-kilometers, roughly one-half of the historic range of the endemic species. However, in this instance, hybrid derivatives did not eliminate the parental types from their habitat; rather, they produced a complospecies of mixed parentage within the parental habitat. Likewise, there are a few reports of local extinction of one or both parents by hybrid-derived lineages in plant species (Baker 1948; Sauer 1957; Kiang et al. 1979).

To our knowledge, our study of *Raphanus* in California represents the first report of biparental extinction by hybridization in plants to be confirmed with genetic evidence.

For the last few centuries, human activities have brought many allopatric taxa into sympatry (Abbott 1992). These sympatric associations might have sometimes caused the local extinction of one or both parental taxa and the evolution of lineages by genetic admixture. The reasons such events are rarely reported are that they might have gone undetected because of the rapid rate with which such evolution progresses (Echelle and Connor 1989; Huxel 1999; Wolf et al. 2001) or because parents and hybrids are difficult to distinguish morphologically from one another (Wiman 1979; Gaskin and Schaal 2002; Snow and Campbell 2005). In the case of *Raphanus*, the historical records of Frost (1923) and Panetsos and Baker (1967), coupled with our morphological and molecular analysis, have captured the dynamic story of bilateral extinction by hybridization and subsequent swampying by the hybrid derivatives.

Wild radish has been widely studied by ecologists and...
evolutionary biologists (e.g., Stanton 1987; Ellstrand et al. 1989; Snow 1990; Mazer and Wolfe 1992; Marshall 1998; Irwin and Strauss 2005). Some of these earlier studies treated wild radish as a feral *R. sativus* escaped from cultivation that became naturalized in California. In light of our findings that wild radish is a genetic mix of two *Raphanus* species, some of the earlier discussions regarding the origin of higher genetic diversity, phenotypic plasticity, flower color polymorphism, and colonizing success in wild radish might become more meaningful by considering wild radish in California as an introgressed lineage. Only future experimental research can determine how the evolution of invasiveness (Snow and Campbell 2005) occurred in this particular successful hybrid lineage.

**ACKNOWLEDGMENTS**

We thank J. Conner, A. Snow, Roskilde University Botanic Garden, the University of Hamburg Botanic Garden, the University of Liège Botanic Garden, and the University of Luca Ghini Botanic Garden for *Raphanus* seeds used in our experiments. We are grateful to L. Rieseberg, R. Whitkus, and C. Ridley for critical reading of an earlier version of this manuscript.

**LITERATURE CITED**


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APPENDIX

The sources of *Raphanus raphanistrum* (nos. 1–2), California wild radish (nos. 3–26), and *R. sativus* cultivar (nos. 27–30) populations for morphological measurements.

<table>
<thead>
<tr>
<th>No.</th>
<th>State/County/Country</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Michigan</td>
<td>pods provided by J. Connor, Michigan State Univ., East Lansing, MI</td>
</tr>
<tr>
<td>2</td>
<td>Denmark</td>
<td>Botanic Garden, Roskilde University, Roskilde</td>
</tr>
<tr>
<td>3</td>
<td>Colusa</td>
<td>east side of Highway (HW) 45, 8 mi N of Colusa. Roadside at the “Reservation Road” sign</td>
</tr>
<tr>
<td>4</td>
<td>Sutter</td>
<td>west side of HW99 between Tudor and Yuba City; Half mi S of Messick Rd</td>
</tr>
<tr>
<td>5</td>
<td>San Joaquin</td>
<td>along HW88, 9.2 mi E of Waterloo, 14.0 mi E of HW99 and 88 intersection</td>
</tr>
<tr>
<td>6</td>
<td>San Joaquin</td>
<td>Escalon; S side of HW120, 2 mi E of Escalon</td>
</tr>
<tr>
<td>7</td>
<td>Stanislaus</td>
<td>Oakdale; roadside waste place on HW120, 4 mi E of Oakdale</td>
</tr>
<tr>
<td>8</td>
<td>Merced</td>
<td>route J9 at Palm, S of Cressey; waste places along railroad tracks</td>
</tr>
<tr>
<td>9</td>
<td>Tulare</td>
<td>Lindcove; both sides of HW198, 1.5 mi E of Mehrton Rd</td>
</tr>
<tr>
<td>10</td>
<td>Riverside</td>
<td>Riverside; vacant lot N of Martin Luther King Blvd., W of Pennsylvania Ave</td>
</tr>
<tr>
<td>11</td>
<td>Riverside</td>
<td>Hemet; surrounding vegetable fields between Palm Ave and Diamond Valley Rd</td>
</tr>
<tr>
<td>12</td>
<td>Alameda</td>
<td>Berkeley; roadside bank near top of Euclid Ave</td>
</tr>
<tr>
<td>13</td>
<td>San Mateo</td>
<td>HW1; 1 mi. S of Pescadero Rd, edge of artichoke field</td>
</tr>
<tr>
<td>14</td>
<td>San Mateo</td>
<td>Half Moon Bay; behind HMB State Beach</td>
</tr>
<tr>
<td>15</td>
<td>San Mateo</td>
<td>Pacifica; around waste places 1 mi S of town and about 100 yards from the beach</td>
</tr>
<tr>
<td>16</td>
<td>Monterey</td>
<td>Greenfield; west side of HW101 between Frontage Rd and Thorne Rd</td>
</tr>
<tr>
<td>17</td>
<td>Monterey</td>
<td>Seaside; E of HW218 at Via Verde Rd, roadside near Frog Pond Nature Reserve</td>
</tr>
<tr>
<td>18</td>
<td>Monterey</td>
<td>Moss Landing; back side of the beach at Moss Landing State Beach</td>
</tr>
<tr>
<td>19</td>
<td>San Luis Obispo</td>
<td>Morro Bay State Park along roadside between beach and golf course</td>
</tr>
<tr>
<td>20</td>
<td>Santa Barbara</td>
<td>SE of Santa Maria, At edges of pastures near the intersection of Palmer and Cat Cyn. Rd</td>
</tr>
<tr>
<td>21</td>
<td>Santa Barbara</td>
<td>HW1 at N of Guadalupe; roadside at the south end of bridge over Santa Maria River</td>
</tr>
<tr>
<td>22</td>
<td>Orange</td>
<td>Huntington Beach; east side of the Pacific Coast HW, S of Beach Blvd</td>
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<td>San Diego</td>
<td>Escondido; N of HW78 and E of Bear Valley Pkwy</td>
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<tr>
<td>24</td>
<td>San Diego</td>
<td>Oceanside; Meyers St., one block from beach near St. Malo</td>
</tr>
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<td>25</td>
<td>San Diego</td>
<td>Encinitas; HW1 across the road from Self Realization Fellowship</td>
</tr>
<tr>
<td>26</td>
<td>San Diego</td>
<td>Imperial Beach; Tijuana River estuary, near Visitors Center</td>
</tr>
<tr>
<td>27</td>
<td>Cherry Belle-1</td>
<td>Better Homes &amp; Gardens Seed Co., red colored hypocotyl and root</td>
</tr>
<tr>
<td>28</td>
<td>Cherry Belle-2</td>
<td>Northrup King Seed Co., red colored hypocotyl and root</td>
</tr>
<tr>
<td>29</td>
<td>Giant White Globe-1</td>
<td>Better Homes &amp; Gardens, white colored hypocotyl and root</td>
</tr>
<tr>
<td>30</td>
<td>Giant White Globe-2</td>
<td>Northrup King, white colored hypocotyl and root</td>
</tr>
</tbody>
</table>