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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*.

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 1996; Huxel 1999; Ferdy and Austerlitz 2002; Levin 2002; Rubidge and Taylor 2004), and as an interesting evolutionary process (Wayne and Jenks 1991; Arnold 1997; Rieseberg et al. 2003; Grant et al. 2004; Taylor et al. 2006). The emphasis has been on gene flow from a locally common taxon to a locally rare one. Theoretical studies have demonstrated that the extinction of a population can occur after relatively few generations of moderate levels of hybridization with another, more abundant population (Huxel 1999; Epifanio and Phillip 2000; Wolf et al. 2001). The process can occur by reproductive interference (Levin et al. 1996) or by the genetic absorption of one population into the other so that the less abundant taxon is essentially replaced by the more abundant one. The latter phenomenon is often termed ''genetic assimilation'' (Ellstrand and Elam 1993; Levin et al. 1996; Rhymer and Simberloff 1996). (Note that genetic assimilation has an entirely different meaning as well; Waddington 1953.) The taxonomic status of the resulting population is sometimes termed a ''compilospecies'' (Harlan and deWet 1963).

Genetic assimilation or extinction can also take place when one or both hybridizing sympatric taxa can be replaced by their hybrid-derived lineages. For instance, if hybrid derivatives have the same or higher fitness than their parents, and if they continuously backcross with one of the parents, eventually the hybrid-derived lineages may cause the local extinction of that parent (Rieseberg and Gerber 1995; Anttila et al. 1998). Additionally, but rarely discussed, is the possibility of extinction of both hybridizing parents by swamping from their hybrid-derived descendants. Consider the following scenario: Assume two previously isolated, cross-compatible taxa come into contact in approximately equal frequencies. Likewise, assume that their hybrids and hybrid descendants suffer no fitness drop relative to their parents. Under simple panmixia and genomic recombination, the frequency of the hybrid derivatives increases over time, eventually replacing the parents. The same result may be obtained if the hybrid derivatives have superior fitness relative to their parents. Provided enough time, the bi-introgressed lineage may evolve into a new taxon that combines the characters of both parents. The intermediate phenotypes and genotypes of the new introgressed lineage might make this lineage appear as a homoploid hybrid species (Rieseberg 1997). However, homoploid hybrid derivatives are quite different from a bi-assimilated lineage. Homoploid hybrid derivatives are direct descendents of first- or early-generation hybrids without subsequent introgression, have strong reproductive isolating barriers relative to both of their parents, and generally establish in novel habitats, rarely causing extinction of parents (Rieseberg 1997; Buerkle et al. 2000).

The paucity of reports of extinction by hybridization may be partly due to the fact that if hybrids do not show reduced fitness, genetic swamping will often proceed so quickly that it will often be missed by scientists (Echelle and Connor 1989; Ellstrand and Elam 1993; Ferdy and Austerlitz 2002).

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. rusticus* 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 substantial 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 tamariscinus* and *A. tuberculatus*, have hybridized so extensively that hybrid derivatives have apparently completely replaced their parents (Sauer 1957). Likewise, the hybrid zone between the stripeseed 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 cytogenetic 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* (here after 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.
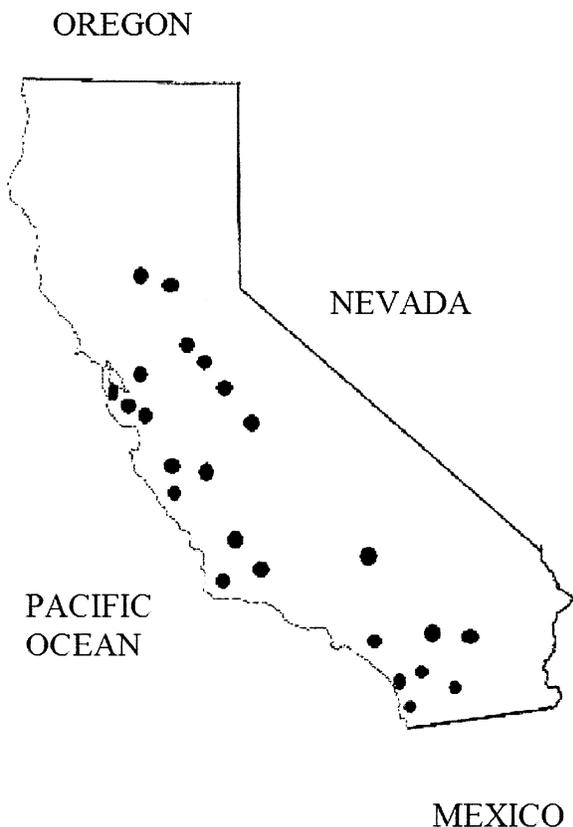
OREGON

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

TABLE 1.   Populations of radish groups used in the allozyme analysis.

Group	Pop. ID	Location
Raphanus raphanistrum	1	Germany, Botanical Garden, University of Hamburgh
	2	Belgium, Botanical Garden, University of Liege
	3	Italy, Botanical Garden, University of Luca Ghini
Wild radish	4	Mendocino Co., California (CA), near Gualala City
	5	Marin Co., CA, near seashore at Point Reyes
	6	Santa Barbara Co., CA, between Twin Oaks Rd and Highway (HW) 192
	7	San Diego Co., CA, NE of San Diego City near state route 76
	8	Colusa Co., CA, E of HW45, 8 mi N of Colusa
	9	Sutter Co., CA, HW99 near Oswald Rd
	10	Sacramento Co., CA, HW104, 10 mi E of HW99
	11	Riverside (1) Co., CA, Near Univ. of California, Riverside (UCR)
	12	Riverside (2) Co., CA, garden site, UCR
	13	Riverside (3) Co., CA, 5 mi S of Hemet
R. sativus	14	Cherry Belle-1, Better Homes & Gardens Seed Co.
	15	Cherry Belle-2, Northrup King Seed Co.
	16	Giant White Globe-1, Better Homes & Gardens Seed Co.
	17	Giant White Globe-2, Northrup King Seed Co.

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* still exist in Europe. Third, the *R. raphanistrum* populations that were the ancestors of wild radishes in California were originally introduced from Europe (Panetsos and Baker 1967). Thus, our inclusion of European jointed charlock in population genetic analysis is congruent 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 1.1.42), 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 Levene (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.

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

Traits[1]	*R. raphanistrum* n[2] $= 20$	Wild radish $n = 229$	*R. sativus* $n = 40$
Bolting date	22.39[a][3] (19.95–25.70)[4]	31.62[b] (29.51–33.11)	48.98[c] (43.65–54.95)
Flowering date	34.67[a] (30.90–38.90)	43.65[b] (41.69–45.71)	69.18[c] (66.07–81.28)
Flower color	yellow	white, purple, pink, bronze, yellow	purple and white
Root length (cm)	11.22[a] (9.95–13.18)	14.45[b] (13.18–15.49)	17.38[c] (14.79–19.95)
Root width (cm)	0.87[a] (0.72–1.06)	1.74[b] (1.58–1.95)	5.12[c] (4.27–6.17)
Fruit diameter (mm)	1.41[a] (0.37–2.46)	6.24[b] (5.71–6.77)	6.10[b] (5.10–7.10)
Fruit shape	15.09[a] (13.48–16.70)	8.90[b] (8.14–9.65)	8.02[b] (6.49–9.56)
Fruit weight (g)	0.11[a] (0.03–0.20)	0.33[b] (0.29–0.36)	0.08[a] (−0.005–0.162)

[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.

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 radish (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

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.

	Function 1	Function 2
A. Traits		
Root width	0.91	−0.46
Flowering date	0.48	−0.24
Root length	0.21	−0.18
Bolting date	−0.36	0.48
Fruit diameter	−0.07	1.07
B. Taxa		
R. raphanistrum	−1.66	−1.94
Wild radish	−0.54	0.20
R. sativus	4.02	−0.24

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 $\lambda = 0.21$, $\chi^2 = 315.22$, $P < 0.000$, df $= 10$; DF_2, Wilk's $\lambda = 0.77$, $\chi^2 = 52.96$, $P < 0.000$, 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.
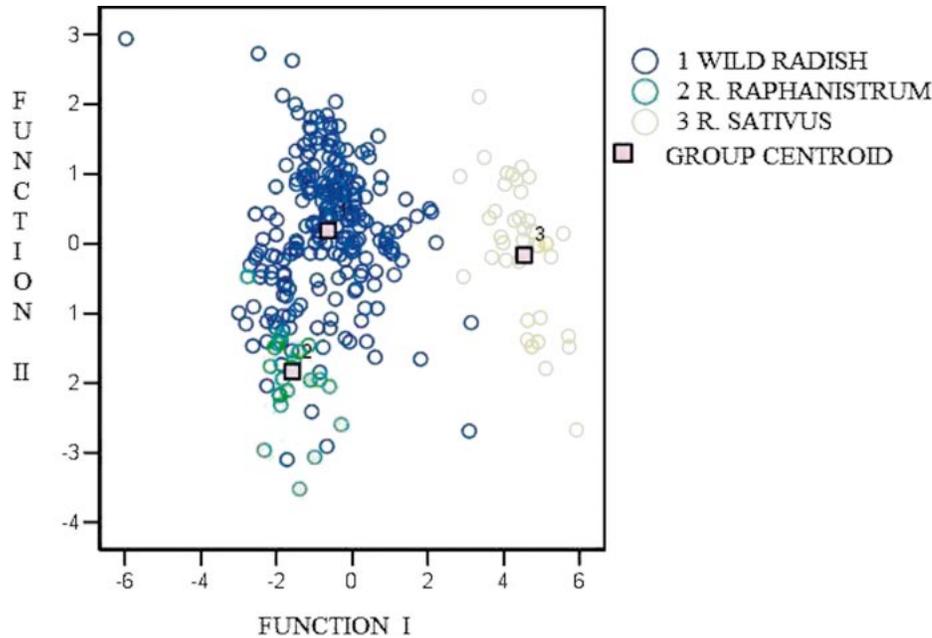
FIG. 2. Plot of the first two axes of discriminant analysis of five morphological measurements from three *Raphanus* taxa.

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 are 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

TABLE 4. Summary of electromorph frequencies at 10 polymorphic loci. *n*, sample size.

Locus	Alleles	R. raphanistrum (n = 74)	Wild radish (n = 368)	R. sativus (n = 80)
Pgm-1	A	0.00	0.02	0.00
	B	0.00	0.22	0.73
	C	0.68	0.57	0.04
	D	0.32	0.19	0.23
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

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 competitively 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 substantial seed bank) is more than sufficient for extinction by hybridization to occur.

Our allozyme data and *structure* analysis (Fig. 3) also support 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, *structure* analysis using the allozyme data for assignment to two clusters reveals the admixed nature of the contemporary California wild radish individuals. Furthermore, the HW equilibrium at loci in mixed populations clearly shows that California wild radish individuals comprise a separate evolutionary unit from their parents (Figs. 2, 3). Interestingly, the allozyme data combined with those discussed above regarding uniformity of morphology suggest that genetic diversity per se does not currently contribute to the success of California 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 populations have unswollen roots and early flowering similar to *R. raphanistrum*. Although wild radishes and cultivars possess 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 enlargement, 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 flowering, 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 adaptation 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 dehisce, they often leave a single seed still attached to the plant, a built-in dispersal polymorphism (D. L. Marshall, J. M. Clegg, and N. C. Ellstrand, pers. obs.). Wild

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.

	Population level estimates (averages of 10 loci)					Taxon level estimates		
	n	H_o	H_e	Polymorphic loci	Loci in H-W equilibrium	H_o (SD)	H_e (SD)	F_{ST}
R. raphanistrum						0.38 (0.23)	0.43 (0.25)	0.22
1. Germany	26	0.42	0.39	9	7			
2. Belgium	27	0.45	0.39	9	8			
3. Italy	20	0.23	0.23	6	6			
Wild radish						0.46 (0.14)	0.51 (0.16)	0.12
4. Mendocino	20	0.48	0.48	10	9			
5. Marin	15	0.44	0.46	10	10			
6. Santa Barbara	18	0.42	0.47	10	10			
7. San Diego	20	0.39	0.41	10	10			
8. Colusa	30	0.40	0.46	10	9			
9. Sutter	31	0.41	0.42	8	8			
10. Sacramento	44	0.48	0.48	10	10			
11. Riverside-1	80	0.46	0.49	10	9			
12. Riverside-2	45	0.46	0.47	10	9			
13. Riverside-3	43	0.44	0.44	10	9			
R. sativus						0.15 (0.14)	0.33 (0.21)	0.54
14. Cherry Belle-1	30	0.09	0.09	6	4			
15. Cherry Belle-2	20	0.12	0.12	3	3			
16. Giant White Globe-1	30	0.15	0.15	3	2			
17. Giant White Globe-2	20	0.30	0.30	8	8			

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 compilospecies 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 swamping by the hybrid derivatives.

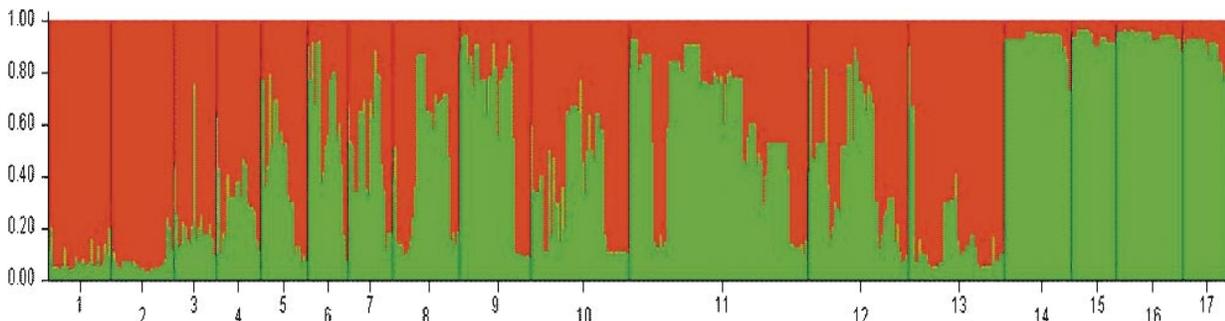Wild radish has been widely studied by ecologists and

FIG. 3. Assignment of individuals to populations (1–17) and to a cluster ($K = 2$) based on multilocus allozyme genotypes. x-axis: populations 1–3, 4–13, and 14–17 are from *R. raphanistrum*, California wild radish, and *R. sativus* respectively (see Table 1 for the details). y-axis: probability of assignment.

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 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 much 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.

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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.

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