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INTEGRATING GENE FLOW, CROP BIOLOGY, AND FARM MANAGEMENT IN ON-FARM CONSERVATION OF AVOCADO (*PERSEA AMERICANA*, LAURACEAE)[1]

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Maintaining crop diversity on farms where cultivars can evolve is a conservation goal, but few tools are available to assess the long-term maintenance of genetic diversity on farms. One important issue for on-farm conservation is gene flow from crops with a narrow genetic base into related populations that are genetically diverse. In a case study of avocado (*Persea americana* var. *americana*) in one of its centers of diversity (San Jerónimo, Costa Rica), we used 10 DNA microsatellite markers in a parentage analysis to estimate gene flow from commercialized varieties into a traditional crop population. Five commercialized genotypes comprised nearly 40% of orchard trees, but they contributed only about 14.5% of the gametes to the youngest cohort of trees. Although commercialized varieties and the diverse population were often planted on the same farm, planting patterns appeared to keep the two types of trees separated on small scales, possibly explaining the limited gene flow. In a simulation that combined gene flow estimates, crop biology, and graft tree management, loss of allelic diversity was less than 10% over 150 yr, and selection was effective in retaining desirable alleles in the diverse subpopulation. Simulations also showed that, in addition to gene flow, managing the genetic makeup and life history traits of the invasive commercialized varieties could have a significant impact on genetic diversity in the target population. The results support the feasibility of on-farm crop conservation, but simulations also showed that higher levels of gene flow could lead to severe losses of genetic diversity even if farmers continue to plant diverse varieties.

Key words: avocado; crop conservation; effective population size; ethnobotany; gene flow; genetic diversity; genetic drift; Lauraceae; *Persea americana*.

The justification for conserving crop genetic diversity is straightforward. Genetic traits that are or may be useful to agriculture are worth conserving, including those that affect disease resistance, environmental tolerance, and taste. Thus, the standard for conserving genetic diversity in crops is perhaps more easily met than that for maintaining population viability in noncrop species.

Following the Green Revolution, researchers documented an abrupt transition in farmers' fields from diverse collections of traditional varieties to a few high-yielding cultivars (Frankel and Hawkes, 1975; Frankel et al., 1995). Millions of dollars have now been spent on seed banks to preserve crop varieties and closely related species (Tanksley and McCouch, 1997) but, in addition to their cost, such ex-situ collections are limited in scope and do not conserve the evolutionary processes that allow crops to adapt to changing environments.

More recent field work showed that many farmers who adopted modern varieties often continued to plant traditional cultivars. Case studies demonstrated such dual strategies with corn (*Zea mays*) in Mexico (Bellon and Brush, 1994), potatoes (*Solanum* spp.) in Peru (Brush et al., 1994), and wheat (*Triticum* spp.) in Turkey (Brush and Meng, 1998). This work provided evidence that on-farm conservation might prove a complementary and, in some cases, an alternative strategy to static collections.

However, important aspects of crop in-situ conservation have not been addressed, such as the consequences of gene flow from genetically uniform cultivars into diverse populations. There is now substantial evidence that gene flow between crops and their wild relatives is widespread (Lee and Snow, 1998; Ellstrand et al., 1999; Jenczewski et al., 1999; Burke et al., 2002; Montes-Hernandez and Eguiarte, 2002). For example, Ellstrand et al. (1999) gathered evidence of gene flow between crops and their wild relatives in 12 of 13 major food crops studied. The opportunity for gene flow between crop varieties of the same species is probably even greater because they are often planted in close proximity (e.g., see Brush, 2000).

The conservation problem is that gene flow from genetically uniform subpopulations could ''swamp'' genetic diversity in traditional crop populations. That is, a field planted with genetically identical crop plants represents a single individual whose collective breeding success in the whole population can be very large. This skewed breeding success can dramatically reduce effective population size (Ryman et al., 1995), creating bottlenecks that lead to rapid losses in genetic diversity even when census sizes are large. The phenomenon is similar to extinction by hybridization (Rhymer and Simberloff, 1996; Ellstrand et al., 1999; Allendorf et al., 2001) but the invasion can come from within the same population. Thus, in terms of allelic diversity, it is a death by dilution.

In the case of avocado (*Persea americana*), the genetic makeup of orchards in Central and South America has changed dramatically in recent years (Smith et al., 1992). A few highly marketable varieties are grafted (vegetatively propagated), creating orchards of genetic clones. On the farms studied, seeds

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from traditional varieties are used as rootstock but their leaf and fruit-bearing branches are cut off when the grafted shoot (scion) is attached. Although clonally propagated, mature grafts are fertile and can pollinate traditional cultivars. Grafting is extensive in Costa Rica, which is one of the centers of domestication for the West Indian race of avocados (var. *americana*; Smith et al., 1992). In addition, avocado may be subject to particularly high levels of gene flow from genetically uniform varieties because of its high outcrossing rate (Whiley and Schaffer, 1994).

This study focuses on three types of avocado trees that are defined by their origins and method of propagation. Grafted trees are the few select varieties that are propagated clonally by grafting techniques. Each graft variety or genotype is typically planted in large numbers. Traditional cultivars are those varieties recognized by farmers as having local origins. A few traditional cultivars are grafted but the term will be reserved for those trees that are propagated from seed and grown on their own roots. The term graft progeny refers to seed-propagated trees that were determined by parentage testing to have at least one graft parent.

We note that most instances of gene flow between grafts and traditional trees will occur in fields where the two trees are planted together. However, because farmers frequently exchange, borrow, or take seed from other farmers, the seedlings growing on a farm are not necessarily the progeny of the trees on the same farm. Our goal was to measure the end result of both the biological constraints on gene flow and any intervening barriers created by farmer's decisions on which seeds to plant. Thus, our strategy was to randomly sample the entire youngest cohort of seedlings to quantify overall gene flow.

Our preliminary research showed that farmers in the study region, the Pacific lowlands of Costa Rica, began widespread grafting within the last 20 yr. Thus, the orchards in the region offered a baseline to compare changes in genetic diversity, with trees over 20 yr old providing a sample of the population before the introduction of grafts and younger cohorts representing the post-grafting population. In addition, there were still towns in the region with few grafted trees, which could be used as control populations. In preliminary tests, these controls had no detectable background difference in population structure between cohorts.

Currently, there are no methods that integrate gene flow, crop biology, and planting practices into quantifiable projections of genetic diversity. Our goal was to measure these factors in one case study and explore their synergistic effects. We first used molecular markers to quantify gene flow between grafted varieties and traditional cultivars. Ethnobotanical data was then collected to analyze farming strategies that could affect genetic drift. For instance, changes in cultivar preference could affect the longevity of grafted trees, which could, in turn, influence genetic drift by altering the lifetime breeding success of clonal genotypes. To integrate management practices and genetic parameters, a Monte Carlo simulation was adapted to track genetic diversity over time by accounting for gene flow, planting practices that affected the life history of trees, and directional selection. The larger goal was to model microevolutionary forces on crop populations and assess long-term crop conservation in situ in one model system.

MATERIALS AND METHODS

Study area—The main study town, San Jerónimo, Costa Rica, is one of the most commercialized regions in the country for avocado production and other fruits. San Jerónimo is in the seasonal forest of Costa Rica's Pacific coast with 2–3 m of rain annually and a 3–4 mo dry season (Holdridge's tropical moist forest life zone; Janzen, 1983). The town is approximately 300 m above sea level (10°00′ W, 84°45′ N). The inhabitants are of European and mixed indigenous-European descent and most farmers owned the land they worked. Their farms were situation along a 2.5-km segment of a dirt road, which was approximately 10 km north of the regional center of Esparza. The putative region of domestication for the West Indian race of avocados (*Persea americana* var. *americana*) is the narrow Pacific Costal plain stretching from Guatemala to Panama (Smith et al., 1992), which includes the study area. San Jerónimo, accessible only by dirt road, is about 80 km northwest of the capital, San José. However, during avocado harvest season (April–July), middlemen regularly come to purchase avocados for the large regional markets in the country's Central Valley, where about 90% of the population lives. A second town where avocados were grown, Londres, had no mature grafted trees and served as a control. It is located approximately 50 m above sea level (9°25′ W, 84°10′ N). Inhabitants were also of European and mixed indigenous-European descent, many of whom worked in the tourist town of Quepos, approximately 20 km to the west.

Demographic survey and sample collection for DNA analysis—First, tree demographic data was collected as follows: avocado trees on 48 farms (about 90% of the farms in San Jerónimo) were inventoried and classified into age cohorts and method of propagation (by seed or grafting). The variety of each grafted tree was also recorded. Farmers were asked to age older trees by using events such as marriages or births (avocado lacks growth rings and the size or girth of trees is not a good indicator of age). Although this dating method was clearly approximate, the primary goal was to choose a set of trees that were planted before most grafted trees were fertile and mature, which was about 10–15 yr before the collections were made. Thus, an estimated age of 20–25 yr left a 10-yr margin of error for collecting a cohort with limited exposure to gene flow from grafted varieties. In addition, the number of each grafted variety (clone) was counted on each farm. To assess the ability of farmers to identify varieties on their farms, seven trees identified by farmers as specific graft varieties were genotyped and checked against genotypes of the founding graft trees. Farmers correctly identified the graft variety in six of seven cases. Leaf and fruit characters were also used to check farmers' identifications and were found to be accurate. Using the inventory as a guide, leaf samples for DNA analysis were collected on randomly selected trees within three age classes (0–1 yr, 0–4 yr, 20–24 yr). Newly flushed leaves were rinsed in 10% bleach for 1 min and dried in silica gel. Samples from each major graft variety were also collected for DNA analysis. The sampling strategy was repeated in Londres, the control town. Data on the varieties and ages of avocado trees was also collected in a third town, Higuito. Herbarium specimens were collected for about 20 varieties, including all major grafted varieties.

DNA extraction and microsatellite analysis—A mini-prep alkyltrimethylammonium bromide (CTAB) extraction was adapted from Doyle and Doyle (1987). Approximately 25 mg of dried plant tissue was macerated in 2-mL Eppendorf tubes using ceramic beads (Bio 101, Carlsbad, California, USA catalog number 6540-424) in a tabletop vortexer. Tissue was then incubated at 60°C in extraction buffer (4% CTAB, 1.4 mol/L NaCl, 20 mmol/L EDTA, 0.1 mol/L T/HC pH 8, and 3% 2-mercaptoethanol). Approximately 50 ng of genomic DNA was used in PCR reactions under standard conditions. Primers for PCR amplification were designed based on the region 50–100 base pairs flanking the microsatellite repeat. Unpublished primers were kindly provided by Michael Clegg (University of California, Riverside, California, USA) except for AVTCT01 and AVAG02 (Sharon et al., 1997). Forward primers were 5′ end-labeled with fluorescent dyes, either 6-FAM, HEX, or TET (Operon Technologies, Alameda, California, USA). Amplified microsatellites were run on an ABI 377 automated sequencer and analyzed with Genescan 3.1 and Genotyper 2.1 (Perkin Elmer, Wellesley, Massachusetts, USA). Primer sequences (5′–3′) are: AVO102F: ttcgccttatcagcgttag, AVO102R: tcttggaaagccctactcc; AVO109F: aactgccttttcttctctatttcag, AVO109R: ggtggggaactgggttagt; AVO128Fb: ccacaaaaatccacaacacaa, AVO128R: cttagccccattcaaatcaac;

AUCR008: tggagcactatgagtccagc, AUCR008R: ccacagttggaacagagtca; AUCR027F: atctgttgtggaggtaatc, AUCR027R: agtaggagcattttagtcc; AUCR035F: gaatgctggcaaaaaaagttag, AUCR035R: tccaatagacaggggctaca; AUCR051F: cccaccaacaaaagcaacaa, AUCR051R: ggagttatgcggaaacgaaaat; AVAG02F: catcatggtgtttgaatgcc; AVAG02R: tggtgaccttaatctaccctcc; AVTCT01F: gattacatccaaggttgg, AVTCT01R: agatcgttccttataccagtgg; AVO129F: aaggctgataagattaggagc; AVO129R: cgtctgggatgagaaagtaa.

Microsatellite data analysis—A set of samples from two different populations was assessed for significant differences in allele frequency distributions using the exact test for population differentiation with the program GENEPOP (Raymond and Rousset, 1995; Goudet et al., 1996). Rarefication methods were used to compare allelic richness (defined as the number of alleles found in a given sample size) in two samples of different size (Hurlbert, 1971). After sample sizes were equalized, changes in allelic richness between samples of two cohorts were assessed over all loci examined using a Wilcoxon's nonparametric paired-sample test (Sokal and Rohlf, 1995).

Parentage rates—The parentage rate is defined here as the frequency of graft haplotype sets found among genotypes in the youngest cohort of trees. A haplotype set is defined as a specific combination of 10 codominant microsatellite alleles from the loci used in the study. Thus, we measured only F1 ''hybrids'' (we did not expect F2 hybrids because of the recent onset of gene flow). To assess the probability of parentage, we used two types of parentage analysis. The first method determined the probability of a graft tree and a sapling (0–4 yr-cohort) sharing at least one allele at all loci. We sought a 95% certainty level that the match in alleles was the only such match in the population of approximately 1600 distinct genotypes. The test accounts for the frequency of the shared alleles in the population and the population size using the following formula (Westneat and Webster, 1994; Dow and Ashley, 1996): $(1 - \Pi_i^L(2p_iq_i + q_i^2))^N$, where q is the frequency of the shared allele, $p = (1 - q)$, N is the population size, and L is the number of loci analyzed. The second method used a likelihood ratio, which compares the likelihood that the candidate in question is the true parent vs. the likelihood that the candidate is an unrelated individual chosen at random from the population (Meagher, 1986). In that test, the likelihood ratio is $T(g_o|g_c)/P(g_c)$, where $T(g_o|g_c)$ is the probability of the offspring's genotype given the candidate parent's genotype and $P(g_c)$ is the population level frequency of the offspring's genotype, which is estimated assuming Hardy-Weinberg equilibrium. For the likelihood analysis, we used the program CERVUS, which also accounts for potential errors in genotyping (Marshall et al., 1998).

The simulation program ManagedPop (Birnbaum et al., 2002) was revised to model the avocado system and to give a selective advantage to one allele. Briefly, the program uses observed microsatellite genotypes as inputs for the allelic composition of two pools of individuals, grafted trees and seed propagated trees. The two pools were further divided into subpools corresponding to breeding types (see later), and each genotype was multiplied by a constant to create a population size of 1600 individuals. Each breeding cycle, alleles were chosen randomly from each breeding pool (A and B) to replace individuals that died in the previous cycle. However, the probability of selecting an allele from one of the eight graft genotypes was determined by its observed parentage rates or other rates specified in the text. Similarly, a selective advantage was given to one allele by assigning it an increased probability of being selected for the genotype of a recruit. The simulations were run with the following other parameters: the age at sexual maturity for grafts was 5 yr and for traditional trees it was 7 yr (maturity ages were determined from interviews with farmers); the number of age classes was 40; the interval at which graft genotypes were switched was 5 (except where otherwise specified in text).

Potential parentage rate—The ''potential parentage rate'' assumed no barriers to gene flow among trees. It was based on the supposition that each physically distinct tree has an equal probability of successfully reproducing and is essentially proportional to the number of distinct graft trees of a single genotype. Avocado is a monoecious diploid with two mating types (A and B). Flowers first open with stigmas receptive, then close and reopen with anthers fully mature, a system that appears to create a barrier to self-pollination (Bergh, 1969). Opposite mating types start the cycle at different times of day (either morning [A type]) or afternoon ([B type]). Graft mating types were determined in the field by observing the presentation of floral parts at different times of the day. The mating types of the major grafts were Nodra = B, Canon = B, Simpson = A, Simpson II = B, Chinchilla = B, Choquette = A, Simmonds = A, Catalina = A. Although ''self-pollination'' has been recorded in single genotype avocado orchards in California (Vrecenar-Gadus and Ellstrand, 1985), we assume here for simplicity that each seed has a type A and type B parent. To calculate a null expectation of free gene flow, the breeding success of a grafted genotype was proportional to the number of physically distinct trees of that same grafted genotype. For example, there were 308 mature trees of the variety Nodra (breeding type B) and an estimated total of 1288 type B trees. Under strict outcrossing, type A trees contribute the same number of gametes but Nodra makes no contribution that pool. Thus, the proportion of Nodra gametes in the entire gamete pool is 12% [308/(2 × 1288)], which represents the expected frequency of its haplotype set in the next generation or its potential parentage rate.

Ethnobotanical data—Farmers on 39 of the 48 farms were interviewed to gather data on their strategies in managing avocados. In formal surveys, farmers were asked about their preferences for graft vs. traditional trees, the reasons behind their preferences, the number of graft and traditional varieties they planted in the past, and how often they switched the grafted varieties they planted. On each farm, the location of trees on farms and the type of field in which avocados were planted was recorded (e.g., orchard, pasture, home garden) and whether grafted trees were planted in the same field as traditional cultivars. In addition, farmers, merchants, and government officials were interviewed informally over 10 mo.

RESULTS

Invasion and coexistence—Inventories showed that 51% of the 3200 mature trees in San Jerónimo were grafted with only five genotypes comprising nearly 40% of all mature trees in the town (Fig. 1). In another Costa Rican town where demographic data was collected, Higuito, about 22% ($n = 594$) of trees inventoried were grafted. In several other towns in the region, farmers appeared to be increasing their use of avocado grafts. From demographic analysis and extensive interviews in the region, the widespread use of grafts began about 20 yr before the study.

Farmers began grafting because avocado seeds, which are usually the result of an outcross, rarely produce fruits similar to the mother tree. Fruit morphology and taste are highly variable (Fig. 2), and many fruits are unmarketable. Thus, San Jerónimo was in the later stages of an apparent trend in which grafted orchards were in the process of creating large pools of genetically homogeneous plants.

However, based on crop demographic data and interviews in San Jerónimo, farmers were not completely abandoning traditional varieties. Grafted varieties began appearing in large numbers in the 15–19-yr-old cohort (39%, $n = 139$), increased in the next two cohorts (64%, $n = 506$, and 71%, $n = 1302$, respectively), and then decreased in the latest 5-yr cohort, in which grafts accounted for only 50% ($n = 754$) of new trees. Several farmers stated that they had switched back from grafted to seed-propagated trees because grafts were shorter lived. In Higuito, farmers also increased their use of grafts in the last 20 yr but the use of grafts also appeared to level off in the most recent cohort. In 5-yr cohorts spanning the last 20 yr from oldest to youngest, the percentage of graft trees was 23% ($n = 13$), 19% ($n = 10$), 35% ($n = 29$), and 30% ($n = 64$), respectively. In San Jerónimo, 47% of farmers interviewed (n

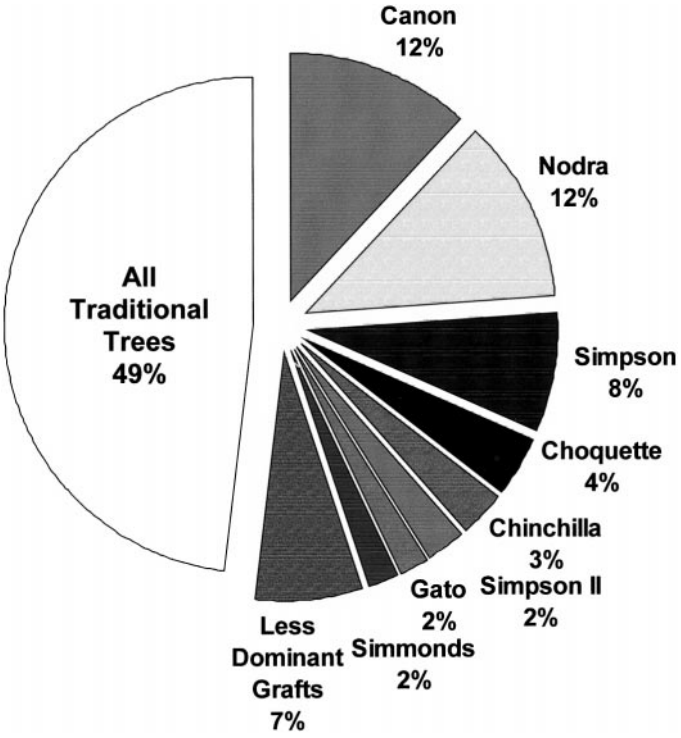

Fig. 1. Percentage of avocado trees (*Persea americana*) that are grafted and seed propagated on farms in San Jerónimo, Costa Rica. Among the grafted trees, each slice represents the percentage of that single genotype (variety) in the entire population. A total of 1683 grafted trees and 1594 traditional trees on the farms were inventoried. (Percentages are rounded.)

= 22 out of 47) preferred grafts because they produced fruit of consistent quality, but 21% of farmers ($n = 10$) said they still preferred the diverse traditional varieties largely because of their hardiness. The remainder of farmers had no clear preference. These data provide support that the coexistence of traditional and grafted trees is likely to continue, providing an opportunity for on-farm conservation but also opportunities for gene flow between grafted and seed-propagated trees.

Population genetics: short-term changes in genetic structure—The recent introduction of grafts in the last 20 years enabled us to ask whether the population had any loss of allelic diversity or other population-level genetic changes since the introduction of grafts. Because historic collections were not available, 10 DNA microsatellite markers were used to compare allele frequency distributions of randomly selected 20–24-yr-old trees ($n = 56$), which were established before the propagation of grafted trees, to randomly selected 0–4-yr-old trees ($n = 88$), which were established 2–3 generations after grafted trees reached reproductive maturity.

The analysis confirmed that the population underwent significant changes in genetic structure. An exact test for population differentiation combining data from all loci showed a statistically significant difference ($P < 0.0015$) in the distribution of allelic frequencies between the 0–4-yr-old cohort and the 20–24-yr-old cohort (POPGEN, Raymond and Rousset, 1995). Such differences would typically distinguish two distinct populations.

A similar set of samples taken in the control town, Londres, where no mature grafts were present, had no significant change

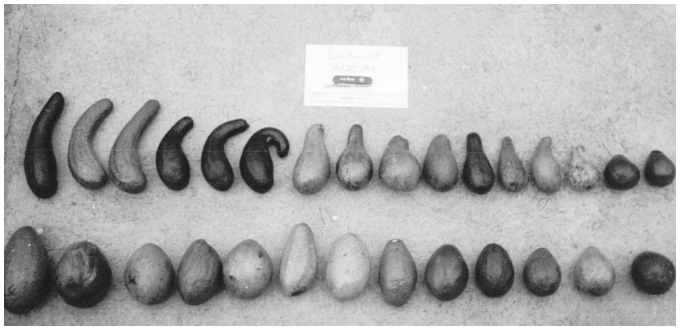

Fig. 2. Traditional avocado (*Persea americana*) fruits gathered on a single day at a market near the study town, San Jerónimo, Costa Rica, in April 1997. High outcrossing rates and the complex genetics of fruit characteristics means the fruit quality of a seed-propagated tree is highly unpredictable. Farmers graft trees to achieve consistency in fruit types.

in allele frequency distributions over the same cohorts using six of the microsatellite markers ($P > 0.18$; $n = 48$, 20–24-yr-old cohort; $n = 49$, 0–4-yr-old cohort). The trends identified with the six markers eliminated the possibility of a significant difference regardless of trends in the remaining markers. Furthermore, differential mortality is not a likely explanation for genetic differences between the cohorts in San Jerónimo because there is no reason to expect neutral markers to be in linkage disequilibrium with loci affecting longevity.

The specific changes in the allele frequency distributions strongly suggested that gene flow from grafted varieties caused the changes in population genetic structure. Allelic diversity, which is defined as the number of alleles in a given sample size, increased in the 0–4-yr-old cohort compared to the 20–24-yr-old cohort in an analysis over all loci (Wilcoxon ranked paired t test, $P < 0.01$; rarefied to equalize sample sizes, see Hurlbert, 1971). Over all 10 loci, the average number of alleles increased from 9.2 to 11.6 in the equalized comparison of 56 individuals. Although *Persea* species do exist in the forests of the region, these close relatives or feral varieties are not a likely source of new genetic diversity because the farms in San Jerónimo are separated from the adjoining forests by large cattle pastures. Furthermore, many of the new alleles detected in the young cohort compared to the older cohort were shared by the grafted varieties. The increase in allelic diversity was due to the fact that the first set of grafted varieties introduced to San Jerónimo appeared to be exotic to the region and carried alleles that were either absent or rare in the original population. For example, of the 41 alleles present in the 0–4-yr-old cohort but undetected in the 20–24-yr-old cohort, 28 (68%) were shared by one of the eight major graft varieties (Appendix I; see Supplemental Data accompanying the online version of this article). Most of these ''new'' alleles were found at low frequencies, suggesting that gene flow from the grafted varieties was moderate. However, gene flow from the genetically uniform graft population had the potential to decrease diversity over longer periods.

Estimating gene flow from grafts—To quantify graft parentage to project long-term effects, we conducted a kinship analysis on the most recent cohort in the population. Several factors made it relatively easy to distinguish grafted trees from seed-propagated trees in the field: (1) farmers usually kept track of which trees were grafted, (2) the grafting scar left between the scion and rootstock was almost always evident,

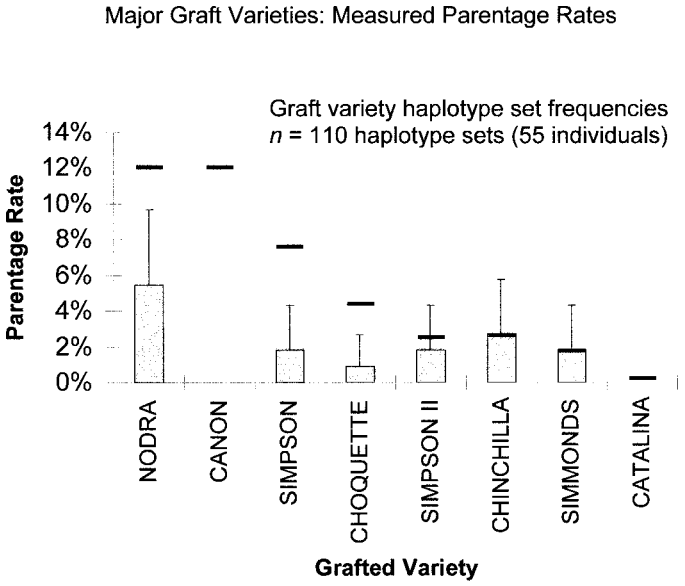

Fig. 3. Parentage rates of the eight major graft varieties of avocado (*Persea americana*) on farms in San Jerónimo, Costa Rica, as determined by parentage analysis. The histogram represents the observed parentage rates. Attached bars indicate the upper 95% confidence interval of parentage rates. Floating bars are the potential parentage rate based on the assumption of free gene flow for all trees of that variety (see Materials and methods).

and (3) in genotyping analyses, a vegetative offspring produced by grafting would carry an identical genotype to a graft mother, a situation that was not observed among the nongrafted saplings that were genotyped.

The basis for parentage analysis was the sharing of a set of codominant markers (i.e., a set of microsatellite alleles) between a randomly sampled sapling and a graft genotype. The genotyping analysis included 88 trees under 5 yr old and the eight major graft genotypes. We analyzed individuals at 9–10 loci to reduce the probability of a false positive match to 5% or less in a population of 1600 individuals. A random sample of adult genotypes was used to estimate population-level allele frequencies (see Materials and Methods). Although some loci did not yield polymerase chain reaction (PCR) products for certain individuals, all seedlings in the young cohorts were either (a) eliminated as potential graft progeny by failing to match an allele with a graft genotype at two or more loci or (b) determined to be graft progeny by matching graft alleles at all loci with a 95% certainty that no other match existed in the population. There was one exception in which a seedling matched a graft genotype at all loci but only had a 78% probability of being the only such match in the population (Appendix II; see Supplemental Data accompanying the online version of this article). The sapling was included as a graft progeny to avoid underestimating gene flow.

A total of 17 graft progeny were found among the 88 trees. However, the last 2 yr of the cohort (55 trees) had the highest rate of graft parentage with 15 of the 55 trees the progeny of one of the eight major graft genotypes (Fig. 3). A total of 16 graft allele (or haplotype) sets were found (one tree had two different graft parents). None of the graft progeny were the result of selfing. Two graft varieties, Canon and Catalina, had no progeny in the sample. The parentage rate, which we define as the percentage of haplotype sets in the sample passed on by graft parents, was therefore 14.5% (16 graft haplotype sets out of 110 haplotype sets sampled).

We calculated the potential parentage at about 47% based on the supposition that the breeding success of a graft genotype was proportional to the number of times it was grafted (see Materials and Methods). The observed parentage rate of 14.5% falls significantly below the potential parentage rate, suggesting that there is a barrier to free gene flow between grafted and traditional trees.

Cultural practices: separating genetic stocks—Farm-level surveys showed an opportunity for gene flow between grafts and the traditional population in the majority of the farms studied. For example, in 31 of the 48 farms studied, farmers planted both grafted trees and traditional trees together in the same field in either a home garden or a mixed orchard. However, while most farmers had mixed graft and traditional tree orchards, the majority of grafted trees were separated from the rest of the population in fields without traditional varieties. In addition, grafted trees were clumped on a few farms. About 85% ($n = 1434$) of all grafted trees inventoried were found on only 21% of farms studied (10 of 48 farms inventoried). The seedlings sampled in the study were treated by farmers as traditional varieties and planted in the same locations. This separation between grafts and traditional trees was caused by the preferences of individual farmers (Fig. 4). For example, among the farmers who listed fruit consistency and rapid maturity as the most important traits in choosing an avocado cultivar, 70% ($n = 1792$ trees) of their orchards were planted to grafts. Among farmers who listed disease resistance and longevity as the most important trait, 98% of their trees ($n = 576$) were traditional varieties. These planting patterns created opportunities for cross-pollination as there were many instances where grafted and traditional trees were within 10–20 m of one another. However, the tendency to segregate the two types of trees generally meant that the nearest neighbor of a traditional variety tree was another traditional variety tree. The small-scale separation of grafted and seed trees may limit pollen flow out of the orchard as avocados are pollinated by small bees, which typically have short pollination distances (Ish-Am and Eisikowitch, 1991). Preferences in the use of seed stock may present other barriers to gene flow. Most farmers said they avoided planting the seeds of grafted trees because they viewed them as being less hardy. This practice appears to hinder a maternal contribution from grafted trees into the population. However, this barrier is apparently leaky because we found that one of the 15 graft progeny had two graft parents.

We cannot rule out that other factors limit gene flow, such as higher male sterility among grafts or outbreeding depression (Allendorf et al., 2001) among graft progeny. Indeed, five the seven major graft varieties were known to have come from breeding stations in the United States and thus, they may have reduced compatibility with the local population. However, the two most popular grafts, Canon and Nodra, were propagated from trees that originated from the local population, according to farmers. These varieties were chosen for grafting because farmers viewed them as better suited to the local environment.

Another planting practice that could influence genetic diversity was the frequent switching of graft varieties, which appeared to be caused by competition for more marketable varieties. This practice could affect genetic drift by limiting the potentially high lifetime breeding success of dominant graft genotypes (Table 1). In the 10–14-yr-old cohort, Simpson

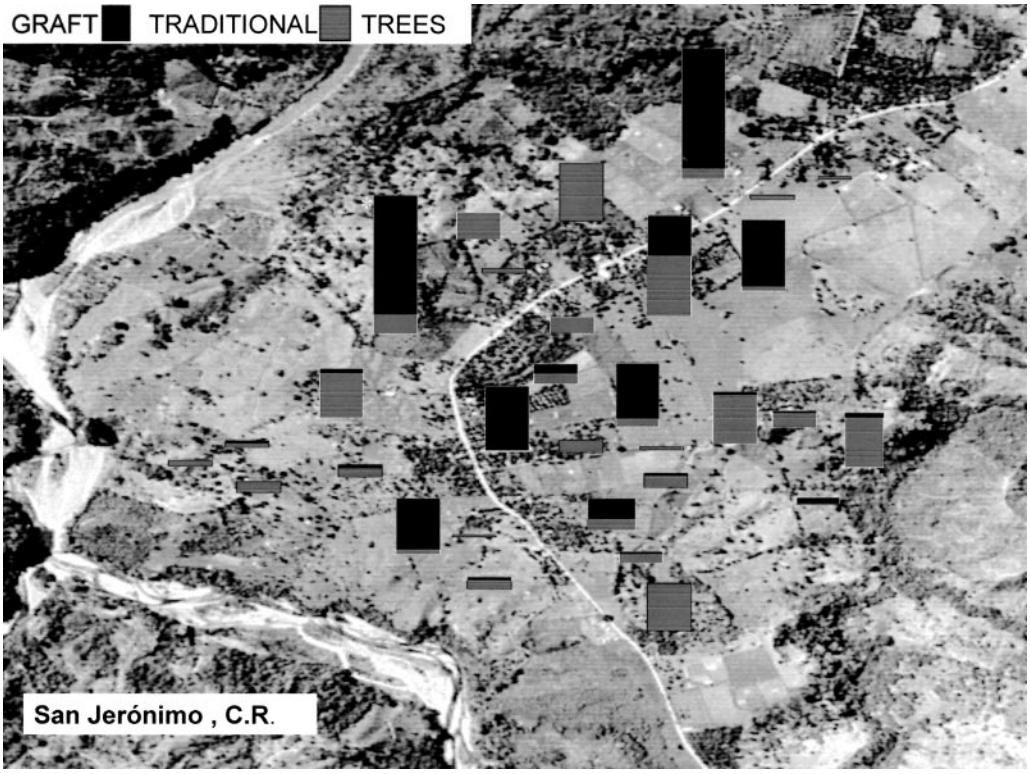

Fig. 4. Areal photo of San Jerónimo, Costa Rica, with each bar proportional to the number of avocado (*Persea americana*) trees found on individual farms. The bars are placed at the map position of the farm they represent and are broken down into grafted or traditional trees. For example, the bar with the asterisk has 222 grafted trees and 35 seed-propagated trees.

was the most popular variety (24% of new grafts, $n = 121$). In the 5–9-yr-old cohort, Nodra (21%, $n = 274$) and Canon (20%, $n = 257$) were the most popular grafts. In the 0–4-yr-old cohort, farmers switched preferences again to a variety called Gato (12%, $n = 92$). The practice of switching graft varieties was incorporated into simulations to project avocado genetic diversity in the simulations that follow.

Simulations: projecting genetic drift on farms—We used the simulation program ManagedPop (Birnbaum et al., 2002) to project the effects of gene flow from the graft varieties on allelic diversity under various management scenarios. In the simulation, the traditional segment of the population ($N = 1600$) produced gametes for the next generation, reproducing annually and maintaining a stable size. Alleles from the grafted trees invaded the gene pool according to rates determined by parentage analysis or other criteria described later. The age-structured simulation allowed us to include management factors that affected life history such as the early maturation and mortality of graft varieties. The effects of switching graft varieties were incorporated by randomly selecting new genotypes from the population to replace highly fertile graft varieties every 5 yr unless noted otherwise. The simulations were seeded with the single-locus microsatellite genotypes of all major graft varieties and genotypes from a random sample of seed propagated trees. Trees were modeled as strict outcrossers.

TABLE 1. The number of trees of each major graft variety of avocado (*Persea americana*) on farms in San Jerónimo, Costa Rica, broken down into age groups.

	Age cohort (years)				
Graft variety	0–4	5–9	10–14	15–19	Total
Nodra	16	274	33	1	324
Canon	45	257	30	17	349
Simpson	7	135	121	0	263
Choquette	2	53	40	17	112
Chinchilla	30	56	10	0	96
Gato[a]	92	0	0	0	92
Simmonds	31	5	39	0	75
Simpson II[b]	40	0	0	0	40

[a] Gato grafts were largely 0–1 yr old, and no progeny of this genotype were found.

[b] Most Simpson II grafts were 3–5 yr old and had reached sexual maturity. Progeny of this genotype were found.

Using the observed parentage rates determined by kinship analysis (14.5%), the simulation projected an average loss of only 1.25 alleles after 150 yr or breeding cycles, which represents a loss of about 6% of the microsatellite allelic diversity detected (Fig. 5a). Random genetic drift alone without graft gene flow caused the loss of almost one allele (0.8 alleles on average) in the same number of breeding cycles (Fig. 5a). Thus, the additional expected loss of diversity due to gene flow was only about 2% of the allelic diversity detected in the sample. Figure 5b shows the loss of allelic diversity over time using the upper and lower 95% confidence limits of observed gene flow rates. The lower allelic diversity curve represents the upper 95% confidence limit on the gene flow rate, which is a worse case estimate leading to an 18% loss of allelic diversity (3.7 alleles) within 150 yr. Simulations run with genotypes from other loci showed similar trends (data not shown).

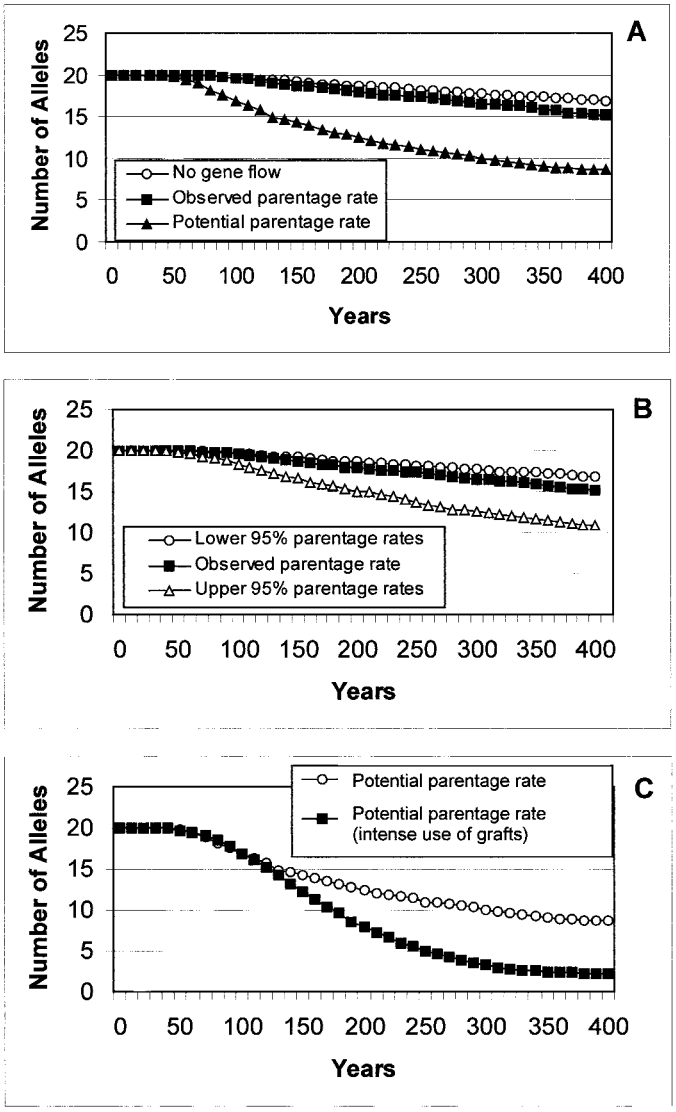

Fig. 5. Simulations of genetic drift in avocado (*Persea americana*) on farms in San Jerónimo, Costa Rica, under various parentage rates and management practices using the Monte Carlo simulation ManagedPop. All curves represent the average of 100 runs.

Simulations indicated that keeping graft gene flow low was the most critical factor in maintaining allelic diversity. Figure 5a shows an allelic diversity curve under higher ''potential'' parentage rates (47%), representing what might happen if farmers abandoned practices that created barriers to gene flow. Under these conditions, about 32% of allelic diversity (6.3 alleles) was lost in 150 yr. The result also suggests that factors that appear to hinder gene flow from grafts are critical in maintaining allelic diversity.

At higher parentage rates, seemingly subtle management practices also have a dramatic effect on the maintenance of allelic diversity. We simulated the higher potential parentage rates (47%) but (a) reduced the number of cultivated graft genotypes from eight to only two varieties and (b) never switched genotypes (Fig. 5c). Under these conditions, 54% of allelic diversity (11.2 alleles) was lost in 150 yr compared to 32% diversity losses at the same rate of graft gene flow but with eight graft varieties and graft stock replacement. The simulation shows how management factors, particularly those dealing with the invasive clonal population, can have a dramatic impact on genetic diversity.

TABLE 2. The response to selection under different graft introgression rates in simulations modeling avocado (*Persea americana*) on farms in San Jerónimo, Costa Rica. Scores are the percentage of 100 replicate simulation runs in which the rarest allele survived 400 breeding cycles. In all cases, the census population size is 1600 individuals.

Selection coefficient[a]	0	0.04	0.08
No gene flow	39%	68%	71%
Observed parentage rate[b]	20%	35%	41%
High parentage rate[c]	2%	3%	4%

[a] The selection coefficient is the selective advantage given to the rarest allele in the locus being tested.
[b] Parentage rate = 14.5% among six genotypes.
[c] Parentage rate = 47% among eight genotypes.

Selection in a managed population—The ability of the avocado farming system to retain selectively favored alleles is critical to adaptation in the farm environment. Factors that decrease effective population size will diminish the efficiency of selection. To explore a case of simple directional selection at a single locus, the simulation was revised to give the rarest allele in the sample a selective advantage, as might be the case for a newly arising favorable allele. The program then recorded how often among replicate simulation runs the favored allele was retained after 400 yr under various management scenarios. Relatively high selection coefficients were used (0.04 and 0.08), which was the range estimated for the *tb1* promoter during maize domestication (Wang et al., 1999).

In the population of 1600 individuals modeled without gene flow from grafts, the favored allele was retained 39% of the time with no selection and 71% of the time with a selection coefficient of 0.08, demonstrating a sharp response to selection (Table 2). When the high potential parentage rate (47%) was simulated, even a selection coefficient of 0.08 was not effective in retaining the favored allele, which survived the 400 breeding cycles only 4% of the time. However, under observed parentage rates (14.5%), the survival of the favored allele increased from 20% with no selection to 41% with a selection coefficient of 0.08. The result shows that the avocado system as managed by farmers could retain its ability to respond to strong directional selection.

DISCUSSION

Moderate gene flow and low diversity losses—By standard benchmarks, a population in which 40% of its individuals were comprised of five genotypes would be at severe risk of genetic erosion. While there was considerable gene flow between grafts and traditional varieties, there appeared to be barriers that prevented much higher potential levels of gene flow. Simulations that incorporated observed stock replacement practices and gene flow rates showed that the loss of genetic diversity over 150 yr was low. Thus, it does not appear necessary to avoid large tracts of uniform varieties or even prevent their gene flow into the diverse population entirely in order to maintain allelic diversity.

We did not analyze what specifically hinders gene flow from grafted varieties. Thus, other factors may contribute to the lower than expected gene flow rates. For example, selection

for grafted varieties with increased fruit set may correlate with lower pollen investment, reducing fertility of grafts. Reduced pollen compatibility is another potential hindrance to gene flow, but the fact that recent grafts were taken from the traditional population suggests that pollen compatibility is an unlikely barrier. In addition, we recorded overlapping flowering times among all trees, including grafts. We also did not observe any measurable differences in gross floral anatomy, and field observations indicated that the same pollinators visited both graft and traditional trees.

On the other hand, practices that led to small-scale separations between grafts and the rest of the population appeared to be plausible explanations for limiting gene flow. Much of the separation was simply due to individual preferences by farmers for one type of tree over another, creating farms dominated by either graft or traditional trees. Thus, a critical factor in maintaining crop diversity may be to encourage farmers to use different cultivation strategies. The results suggest a link between the diversity in crop populations and preservation of cultural systems that permit varied farming strategies. However, because other factors cannot be ruled out, further studies are needed to quantify the extent to which physical separation achieved by different planting patterns affects gene flow between subpopulations (e.g., Vrecenar-Gadus and Ellstrand, 1985).

One important result was that, in addition to the level of gene flow, management practices that influenced the effective size of the grafted population could have a critical impact on genetic drift and diversity. The smaller the effective size of the graft population, at a fixed rate of gene flow, the greater its impact on genetic drift. At the relatively moderate gene flow rates observed, the effect was negligible. However, at higher rates of gene flow, practices pertaining only to the cultivation of uniform plantings, such as the number of varieties used and their replacement frequency, had a severe impact on allelic diversity. Decreasing the number of varieties used and increasing the interval between replacement led to more dramatic losses of genetic diversity. In effect, a large grafted population could harbor a good deal of the population level diversity. The result suggests that only measuring gene flow from grafts or other uniform populations may not be sufficient to assess their impact on genetic drift in target populations.

Importantly, the management of the genetically uniform graft population is strongly influenced by economics. For instance, avocado farmers were highly motivated to plant varieties that were popular in local markets. A competitive atmosphere led to highly coordinated shifts in the varieties planted, ensuring that only a few varieties dominated any individual cohort. This reduced the effective size of the graft population and is probably an aspect of in-situ crop conservation that requires close monitoring.

Still, while farmers managed their crops to maximize profit and minimize crop failure, they created a system that appears capable of conserving much of its genetic diversity. Simulations showed that the avocado population could potentially evolve under selection in response to environmental changes. The result also suggests that selection could effectively oppose gene flow from invasive varieties and help retain traits under selection in the diverse population. Jenczewski et al. (1999) reached a similar conclusion in a study that measured gene flow between domesticated *Medicago sativa* and a wild relative. They found that neutral markers introgressed freely but quantitative traits between the two populations remained distinct. The resilience of these farming systems even under pressure from gene flow underscores a wide potential for in-situ conservation in cropping systems that might not appear ideal at first glance.

Potential crop management changes—Our simulations show that subtle changes in the way farmers manage their avocado orchards can greatly affect genetic diversity. This suggests that in-situ conservation requires monitoring. The study offers a guide to track important changes in planting practices. Inventories of traditional and uniform varieties and baseline measures of gene flow are a first step. Monitoring could also include key management practices: Have farmers changed their spatial planting patterns in ways that break down barriers to gene flow between genetically uniform and diverse subpopulations? Within the genetically uniform segment of the population, are farmers switching from several widely planted varieties to only a few? How often are they replaced? From what population do replacements come? These factors can all be combined in simulations that project drift over long periods.

Incentives to cultivate traditional varieties are an obvious way to aid in-situ conservation. In Costa Rica, we observed that large regional agricultural fairs were excellent markets for traditional varieties. Indeed, aspects of commercial markets can actually enhance genetic diversity (e.g., Brush et al., 2003). For example, many farmers in the study stated that they cultivated several graft varieties instead of one to have fruits that matured at different times, which extended the harvest season and enabled them to take advantage of higher prices later in the harvest season. In addition, our results strongly suggest that agricultural extension services that encourage spatial separation of uniform and traditional varieties would reduce gene flow and decrease genetic drift. Field work also showed that, paradoxically, teaching many farmers instead of just a few to graft appeared to promote experimentation, leading to greater varietal diversity in the graft subpopulation. This would appear to increase the effective size of the invasive population and reduce its impact on genetic drift, assuming genetic diversity within the grafted population also increased.

Conclusions—The crops at greatest apparent risk for diversity losses as a result of gene flow are tropical fruit trees like avocado with high outcrossing rates and relatively small population sizes. However, even annual crops with lower outcrossing rates may be at risk for increased drift from gene flow because discrete generations and annual breeding cycles would lead to smaller effective population sizes compared to long-lived populations. For instance, simulations showed that an annual species of 10 000 individuals with discrete generations and a 5% parentage rate from two genotypes could lose half its genetic diversity in about 100 yr.

One reason why in-situ conservation may be more pressing for tropical fruits is that their seeds do not store well, forcing the maintenance of crops as living collections. In Costa Rica, two living collections of avocado cultivars were both completely destroyed by disease epidemics by the late 1990s (C. L. Loría, University of Costa Rica, personal communication). New collections were subsequently restocked from farms, making farms the repository for ex situ conservation. With any crop, the susceptibility of static collections housed in one or a few locations is a significant concern. For example, in barley, genetic diversity in ex-situ land race collections decreased over time apparently due to bottlenecks caused by the periodic re-

generation of the seed stock (Parzies et al., 2000). The estimated effective population size for these collections was less than five individuals, which is much lower than even the worst case scenarios modeled in this study. Moreover, the potential benefits of growing crops on farms where they can evolve under new conditions such as changing climate has been noted (Brown, 2000).

The management of crops by farmers over millennia has created the diverse populations that are the basis for our food supply. It follows that active management will continue to shape the genetic structure of crops in the future and that in situ conservation planning must account for it. We have developed a first-generation methodology to address the complexities of conserving crops in situ by integrating factors such as gene flow and the management of commercial varieties in terms of genetic drift. On-farm conservation of crop genetic resources is feasible, but the complexities of such a conservation system require justification based on interactions between population dynamics and relevant management practices.

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