Outcrossing between 'Bacon' Pollinizers and Adjacent 'Hass' Avocado Trees and the Description of Two New Lethal Mutants

Raymond J. Schnell[1], Cecile L. Tondo, J. Steven Brown, David N. Kuhn, and Tomás Ayala-Silva
U.S. Department of Agriculture, Agricultural Research Service, Plant Genetics, 13601 Old Cutler Road, Miami, FL 33158

James W. Borrone
Department of Botany, 104 Life Sciences East, Oklahoma State University, Stillwater, OK 74078

Thomas L. Davenport
Tropical Research and Education Center, University of Florida, 18905 SW 280 Street, Homestead, FL 33031

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Abstract. **Avocado (*Persea americana* Mill.) has an unusual flowering mechanism, diurnally synchronous protogynous dichogamy, that promotes crosspollination among avocado genotypes. In commercial groves, which usually contain pollinizer rows adjacent to the more desirable commercial cultivars, the rate of outcrossing has been measured with variable results. Using microsatellite markers, we estimated outcrossing in a commercial California 'Hass' avocado orchard with adjacent 'Bacon' pollinizers. Seedlings grown from mature harvested fruit of both cultivars were genotyped with five fully informative microsatellite markers and their parentage determined. Among the 919 seedlings of 'Hass', 688 (75%) were hybrids with 'Bacon'; the remaining 231 (25%) seedlings were selfs of 'Hass'. Among the 850 seedlings of 'Bacon', 382 (45%) were hybrids with 'Hass' and the remaining 468 (55%) seedlings were selfs of 'Bacon'. The high outcrossing rate observed in the 'Hass' seedlings was expected, because adjacent rows of opposite flowering types (A versus B) are expected to outcross. However, the high selfing rate in 'Bacon' was unexpected. A previous study in Florida using the cultivars 'Simmonds' and 'Tonnage' demonstrated differences in outcrossing rates between complementary flowering type cultivars. In both Florida and California, the A type parents ('Hass' and 'Simmonds') had similar outcrossing rates (≈75%); however, the B type parents ('Bacon' and 'Tonnage") had highly skewed outcrossing rates of 45% and 96%, respectively. Two new avocado lethal mutants were discovered among the selfed seedlings of 'Hass' and 'Bacon'. These were labeled "spindly" and "gnarly" and are similar in phenotype to mutants described in *Arabidopsis* and other crop species.**

Avocado is a subtropical tree that is native from Mexico to northern South America. Avocado was introduced into Florida in 1833, California in 1848, and Hawaii in 1855. Southern California is a major avocado production area in the United States, producing 90% of the total crop, whereas Florida produces 9% and Hawaii 1%. Total domestic production for 2005/2006 was 247,000 tons (Agricultural Marketing Research Center, 2009).

Avocado possesses an unusual flowering mechanism, diurnally synchronous protogynous dichogamy (DSPD), in which the male and female parts of the perfect flower are functional at different times (Davenport, 1986; Ish-Am, 2005). Cultivars are classified based on their flowering behavior as either Type A or Type B; the types differ in timing of male and female function (Stout, 1933). Flowering Types A and B complement one another because when one is functionally female (Stage 1), the other is functionally male (Stage 2). DSPD is thought to promote outcrossing between avocado trees of opposite flowering types (Borrone et al., 2008; Degani et al., 1997), but selfing is frequently observed, so factors such as outcrossing rate and the impact of individual pollen donors must also be considered with regard to fruit set and yield. Commercial avocado groves

often contain both A and B type cultivars with similar flowering periods. This planting method has been recommended as a way to ensure high yields in commercial groves (Bekey, 1986; Gustafson and Bergh, 1966; Robinson, 1933). The necessity of interplanting cultivars of opposite flowering type has been much debated among avocado researchers. Using molecular markers, outcrossing has been demonstrated in many studies; however, the rate varies with location, orchard design, developmental stage of the fruit sampled, and the cultivars used as pollen donors (Ashworth et al., 2007; Chen et al., 2007; Davenport, 1999; Davenport et al., 1994, 2007; Degani and Gazit, 1984; Degani et al., 1989, 1997; Vrecenar-Gadus and Ellstrand, 1985).

Borrone et al. (2008) used microsatellite markers on Florida avocados and demonstrated that when two complementary cultivars are planted together in almost equal numbers, most of the harvestable fruit result from crosspollination. They screened a large population of seedlings germinated from mature fruit collected from a commercial orchard in Miami–Dade County, FL, interplanted with two cultivars of complementary flowering types, 'Simmonds' (A) and 'Tonnage' (B). In the study, 96.1% of the seedlings of 'Tonnage' and 73.8% of the seedlings of 'Simmonds' were found to be outcrossed based on eight microsatellite markers. A similar result was found in California using 10 microsatellite markers to genotype all progeny from a single tree of 'Gwen'. Almost all of the progeny tested (203 of 204) were the result of outcrossing (Ashworth et al., 2007; Chen et al., 2007). Outcrossing and yield have been found to be significantly correlated for a number of avocado cultivars in Israel (Degani et al., 1989, 1997); however, studies in California estimated no correlation or a very weak correlation between outcrossing and yield (Kobayashi et al., 2000; Vrecenar-Gadus and Ellstrand, 1985). Most recently, Garner et al. (2008) estimated outcrossing using 10 6-year-old 'Hass' trees in a commercial grove in California with a single fully informative microsatellite marker. Outcrossing rates were found not to be related to yield or alternate bearing.

'Hass' is the most important avocado cultivar worldwide (Ashworth et al., 2007) and growers receive a premium price for 'Hass' fruit. Interplanting with complementary flowering types reduces the number of 'Hass' trees per hectare and provides the grower with inferior fruit from the other cultivar and thus reduces income. Our objectives in this study were to sample mature marketable fruit from two adjacent rows of 'Hass' (A) and 'Bacon' (B) in a commercial avocado grove in California and determine the rate of outcrossing between them using microsatellite markers. We then compared outcrossing rates in this southern California location and the southern Florida location sampled previously (Borrone et al., 2008) to evaluate the influence of different environmental conditions and genetic backgrounds.

Materials and Methods

Fruit. Marketable avocados were sampled from a mature commercial orchard (Limoneira Company, Santa Paula, CA; lat. 34°18′41″ N, long. 119°08′13″ W, elev. 76.8 m). The orchard is 16.67 ha and planted with 72 rows of 'Hass' trees bordered by one pollinizer row of 'Bacon' on the east and west sides of the orchard. Tree spacing is 6.01 × 6.01 m between and within rows, which is typical for commercial groves in this part of California. 'Hass' rows consisted of 62 trees. 'Bacon' rows consisted of 112 trees spaced at 3.66 m on the west and 54 trees spaced at 6.01 m on the east. Mature marketable fruit were harvested from 12 trees in the west row of 'Bacon' (1027 fruit) and from 12 trees in the adjacent row of 'Hass' (1003 fruit). Fruit was harvested by the grower on 20 Feb. 2007 and seeds were separated from the fruit on site after 1 week of storage. The seeds were packaged and air-freighted back to the Subtropical Horticulture Research Station (SHRS) in Miami, FL. Climatic conditions during the flowering period of 'Hass' and 'Bacon' in 2006 and the historical monthly averages from 2004 through 2008 for February through May (Weather Underground history for Camarillo, CA) are presented in Table 1. The weather station is located at Temple Avenue and Ponderosa Drive (KCACA-MAR4; lat. 34°13′52″ N, long. 119°1′47″ W, elev. 58 m). This station is ≈13.20 km from the Limoneira groves.

Germination. A total of 1003 'Hass' and 1027 'Bacon' seeds were planted. The seeds were planted in 3.8-L plastic pots and allowed to germinate. We used a soilless medium consisting of 50% pine bark, 10% sand, 40% coir dust, 4.5 kg·m⁻¹ of dolomite, and 1.5 kg·m⁻¹ Micromax (Nursery Mix; Atlas Peat & Soil, Boyton Beach, FL) and fertilized with 9 g per pot of Nutricot Total Type 180 (18N–2.6P–6.6K; Florikan E.S.A. Corp., Sarasota, FL). Before use, the soilless medium was steamed for 6 to 7 h until a temperature of 82 °C was reached, allowed to cool, then steamed an additional 2 h. The seeds were irrigated automatically two times daily for 30 min at a rate of 26.6 L/hr.

Sampling. DNA was isolated using the FastDNA® SPIN Kit (MP Biomedical, Solon, OH) as described by Schnell et al. (2003) from young, fully expanded, green leaves collected from the seedlings. Leaves were also collected from the maternal parent trees, 'Hass' and 'Bacon', in California.

Genotyping of progeny. The progeny were screened with five microsatellite markers (Table 2). These markers were developed by Borrone et al. (2007, 2009) or Sharon et al. (1997) and were specifically selected because 'Hass' and 'Bacon' do not share common alleles.

Polymerase chain reactions (PCRs) were prepared following the procedure of Schnell et al. (2003) for primer AVAG25 and Borrone et al. (2008) for all other primers with the following exceptions: 4 ng template DNA was added for each reaction and Taq DNA polymerase with ThermoPol Buffer (New England Biolabs, Ipswitch, MA) was used. PCRs were prepared with a MultiPROBE II PLUS HT EX Robot Liquid Handling System (PerkinElmer, Life Sciences, Downers Grove, IL) and performed on DNA Engine Tetrad 2 PTC-225 thermalcyclers (MJ Research, Waltham, MA). One microliter of the PCR product was combined with 20 μL of a size standard dilution [1:200 ratio of ROX-labeled GeneScan™-400HD molecular size standard (Applied Biosystems, Inc., Foster City, CA) to water], denatured at 95 °C for 30 sec, and chilled on ice. Capillary electrophoresis of the PCR products was performed on an ABI Prism® 3730 Genetic Analyzer (Applied Biosystems, Inc.), and analyzed using GeneMapper® v4.0 (Applied Biosystems, Inc.). Progeny were determined to be the result of self-pollination if only maternal alleles were found at all loci and the result of an outcross between 'Hass' and 'Bacon' if each locus contained an allele from each of these parents. The results were determined using CERVUS v3.0 (Marshall et al., 1998; Slate et al., 2000). After generating an allele frequency data set, this software uses a simulation program to generate log likelihood scores and provides a confidence statistic for assigning paternity. The percentage of expected outcross and selfed progeny was calculated assuming that each individual maternal tree had an equal probability of pollination from each of the pollen donors, including itself.

Results

Cross- versus self-pollination. Of the 2030 seeds collected, 1883 (93%) germinated and 1782 (95%) of these were assayed with the microsatellite markers (Table 3). The 101 individuals not assayed either died before sampling (n = 44) or their DNA extraction or amplification was unsuccessful (n = 57).

The five microsatellite markers used were fully informative (i.e., each parent contained different alleles). All 24 trees from which fruit were harvested were genotyped with the microsatellite markers for trueness-of-type and all were correctly identified. As was expected from the allelic configuration of the parents at these five loci, 12 different alleles were detected in the progeny (Table 2). In addition to the five markers assayed in this study, 104 more microsatellite loci were tested in a single representative each of 'Hass' and 'Bacon'. 'Hass' was found to be heterozygous at 61 loci (56%), whereas 'Bacon' is heterozygous at 64 loci (59%) of the 109 assayed (unpublished data).

The total number of seedlings assayed was 1782; both parents were positively identified for 1769 (99%) of these seedlings. Thirteen individuals were excluded because they contained nonparental alleles, could not be identified as a hybrid or self, or were found to have the incorrect maternal parent. Of the 919 seedlings in which 'Hass' was the maternal parent, 688 (75%) were found to be full-sibs of 'Hass' × 'Bacon'. The remaining 231

Table 1. Monthly mean values for air temperature and total measured precipitation from February through May 2006 (the flowering period of 'Hass' and 'Bacon' avocados in Southern California) compared with mean monthly temperatures and precipitation from 1999 through 2008 given in parentheses.[z]

Month	Avg air temp (°C)			Absolute air temp (°C)		Precipitation (mm)
	Daily	Maximum	Minimum	Maximum	Minimum	
February	13.3 (12.7)	20.0 (18.1)	6.1 (7.1)	31.1	1.7	3.3 (3.1)
March	11.1 (13.6)	16.7 (19.3)	5.0 (7.8)	28.3	0.0	0.51 (1.1)
April	13.9 (14.4)	18.9 (20.1)	8.9 (8.6)	27.2	4.4	0.0 (0.5)
May	16.7 (16.5)	20.6 (21.5)	12.2 (11.5)	26.1	7.2	0.0 (1.1)

[z]Weather measurements were made in Camarillo, CA, ≈13.20 km from the Limoneira groves.

Table 2. Description of five fully informative microsatellite loci used for estimating outcrossing in progeny of a reciprocal cross of 'Hass' and 'Bacon' avocado.

Microsatellite locus	Source	T_a (°C)	Genotype (bp) 'Hass'	Genotype (bp) 'Bacon'
AVAG25	Sharon et al., 1997	50	106	102
SHRSPa099	Borrone et al., 2007	55	76	89
SHRSPa157	Borrone et al., 2009	55	239	223, 233
SHRSPa214	Borrone et al., 2009	55	233	236
SHRSPa327	Borrone et al., 2009	55	118	114, 132

Table 3. Germination results for seed collected from 'Bacon' and 'Hass' avocado fruit and the number of progeny that resulted from outcrossing or self-pollination as determined by genotyping at five microsatellite loci.

Maternal parent	Number of offspring assayed	Number of offspring excluded	Number of outcrosses Observed (%)	Number of outcrosses Expected[z] (%)	Number of selfs Observed (%)	Number of selfs Expected[z] (%)	χ^2
'Hass'	924	5	688 (75)	460 (50)	231 (25)	460 (50)	227.01**
'Bacon'	858	8	382 (45)	425 (50)	468 (55)	425 (50)	8.65**
Total	1,782	13	1,070 (60)	885	699 (40)	885	77.76**

[z]The percentage of expected outcross and selfed progeny were calculated assuming that each individual parental tree had an equal probability of pollinating any other individual tree, including itself.
$\chi^2 = 6.64$, df = 1, $P = 0.01$.

seedlings were selfs of 'Hass'. For the 850 seedlings in which 'Bacon' was the maternal parent, 382 (45%) were found to be full-sibs of 'Bacon' × 'Hass', whereas 468 (55%) were selfs of 'Bacon' (Table 3). All parental assignments were made with 95% confidence (as defined by the delta scores).

Description of lethal phenotypes among the selfed progeny from 'Hass' and 'Bacon'. Among the seedlings of 'Bacon', an unhealthy mutant phenotype was detected for 124 of the seedlings that germinated. Only 112 of the 124 were successfully genotyped and all of these were found to be selfs of 'Bacon'. In total, 468 seedlings resulted from self-pollination of 'Bacon' of which 112 (24%) had the mutant phenotype. This phenotype, which had small, deformed leaves, distorted shoot tips, and a stunted growth habit, was given the name "gnarly" (Fig. 1). These plants died quickly in the greenhouse and could only be kept alive by placing them in deep shade. Even these highly shaded plants slowly died over a 12-month period, resulting in 100% mortality for these mutants. The segregation of this lethal mutant is not inconsistent with that of a single locus, in which the recessive homozygote results in the mutant phenotype with 'Bacon' being heterozygous [χ^2 (3:1) = 0.0514, $P > 0.60$].

A different lethal mutant phenotype was detected among the seedlings of 'Hass'. These 126 plants were small and chlorotic when compared with the normal-looking phenotype and all of them died within 3 months (100% mortality). This phenotype, which had chlorotic leaves and accelerated leaf abscission in comparison with normal plants, was given the name "spindly" (Fig. 2). One hundred six of the 126 "spindly" mutants were successfully genotyped and all were found to be selfs of 'Hass'. Twenty of the "spindly" mutants were not genotyped, because the seedlings were discarded after they died and before tissue samples were collected for DNA extraction. Of the 231 seedlings arising from self–pollination, 46% (106) had the mutant phenotype. The segregation did not fit a single locus model in which the homozygous recessive allele is responsible for the phenotype as was the case for the self seedlings of 'Bacon'. None of the F_1 hybrids of 'Hass' × 'Bacon' or the reciprocal had the "spindly" or "gnarly" phenotype.

Discussion

In this study, 75% of the seedlings collected from 'Hass' trees were outcrossed with 'Bacon' and the remaining 25% were the result of self-pollination. Kobayashi et al. (2000) sampled 'Hass' rows in three California locations immediately adjacent to pollinizer rows of 'Bacon'. The level of outcrossing detected in 'Hass' was 33% in Riverside, 18.2% in Ventura, and 18.7% in Santa Barbara. The difference between the two studies could be the result of the experimental design (12 trees from adjacent rows with 919 fruit versus two trees from adjacent rows with 20 fruit per tree) and the type of markers used
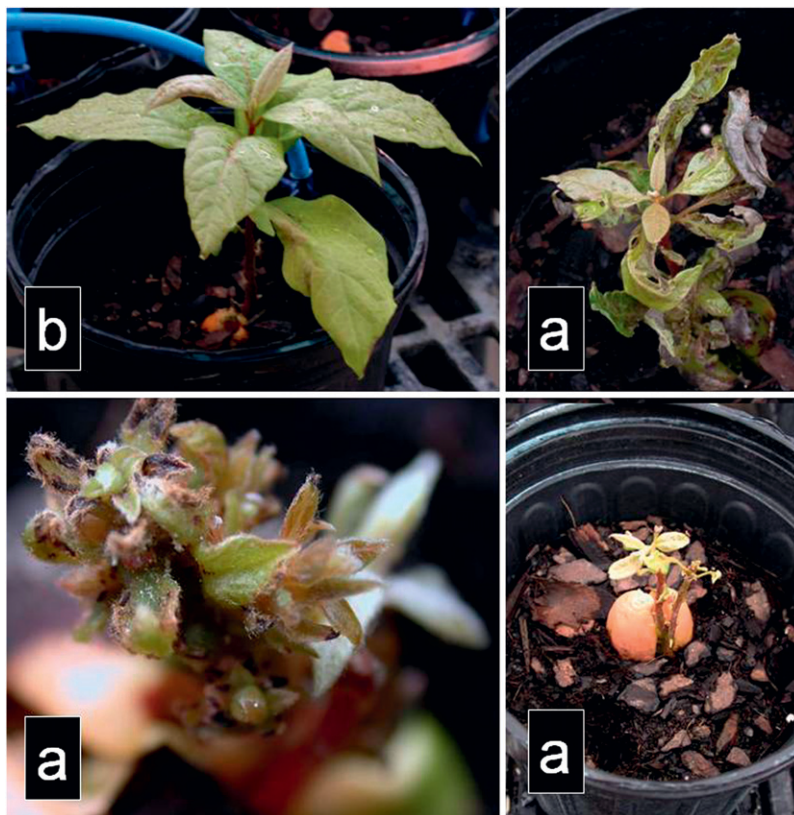

Fig. 1. Mutant phenotype "gnarly" (**A**) resulting from self-pollination of 'Bacon' compared with a normal plant (**B**).
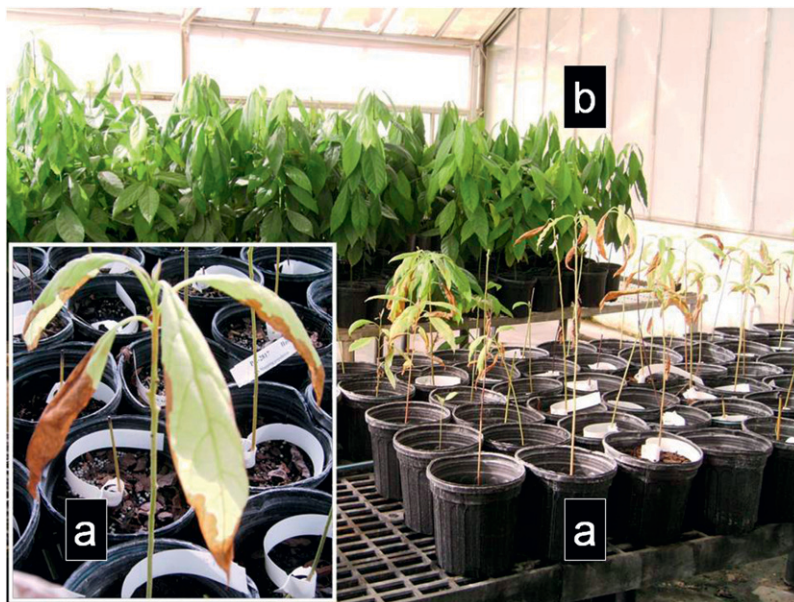

Fig. 2. Mutant phenotype "spindly" (**A**) resulting from self-pollination of 'Hass' in foreground compared with same-age, normal phenotype (**B**) in background. Inset: mutant phenotype (**A**).

(microsatellite versus random amplified polymorphic DNA). Our results are more similar to the results obtained by Vrecenar-Gadus and Ellstrand (1985), in which 89.6% outcrossing of 'Hass' planted with pollinizers was found using two isozyme markers. Likewise, using 14 isozyme markers, Gardiazabal and Gandolfo (1995) found 84.5% to 95%

crosspollination in 'Hass' fields interplanted with pollinizers and 64% crosspollination in 'Hass' fields that were planted as solid blocks. These results are also in agreement with those found by Borrone et al. (2008) from a commercial grove in Florida where 96% of 'Tonnage' and 74% of 'Simmonds' progeny were the result of outcrossing. By contrast, when

'Bacon' was the maternal parent, only 45% of the seedlings resulted from hybridization with 'Hass'; the other 55% were selfs of 'Bacon'.

The asymmetry of outcrossing and selfing rates in these two clones collected from adjacent rows was unexpected. Observations of 'Hass' in research plots in Ventura County indicate that overlap of female and male floral stages occurs when weather conditions are cool (M.L. Arpaia, personal communication), and this overlap has been experimentally demonstrated for both 'Hass' and 'Bacon' (Sedgley and Annells, 1981; Sedgley and Grant, 1983). 'Bacon' can be unpredictable in its flowering behavior, and the period of floral anthesis may not always coincide with that of 'Hass' (M.L. Arpaia, personal communication). Climatic factors that affect avocado production include mean low temperatures during flowering, extreme high temperatures during fruit set, and frost. According to Bergh (1969), under California conditions, the two complimentary flowering types, A and B, display typically normal flowering times when the average temperature (night minimum and day maximum) is above 21.1 °C. As temperatures fall, the daily openings and closings become delayed and irregular, and a single tree may have flowers in both female and male stages at the same time, especially in conditions in which temperatures are cool in the mornings and warm in the afternoons. The fruit that was harvested in Feb. 2007 at the Limoneira grove in Santa Paula resulted from pollination events that occurred in late February through mid-May of 2006. The only unusual temperature patterns recorded during that period were a freeze on 12 Mar. and a cooler than normal minimum average temperature in March (5.0 versus 7.8 °C) (Table 1). One explanation for our results is that during the floral flushes that occurred in 'Bacon', at least one and maybe more did not correspond to a floral flush in 'Hass'. This noncoincidental flowering period could have increased self-pollination among the 'Bacon' seedlings. Alternatively, with increasing evidence indicating that avocado flowers are wind-pollinated (Ying et al., 2009), the direction and distance of pollen drift from one cultivar to the other during the warm periods could also impact the proportions of self- versus crosspollination in the two cultivars. Differential spacing (3.66 versus 6.01 m) between the 'Bacon' and 'Hass' trees could also have affected the amount of selfing because more overlapping branches are known to increase close pollination (selfing in this case). Asymmetries in proportions of cross- versus self-pollinations were also found in a Florida orchard interplanted with complementary cultivars. Borrone et al. (2008) reported 4% of progeny of 'Tonnage' versus 26% of the progeny of 'Simmonds' resulted from self-pollination. In south Florida, 'Simmonds' and 'Tonnage' usually have completely coincidental periods of floral anthesis, and low temperatures do not limit development like in California.

Plant species that have genetically controlled self-incompatibility (SI) mechanisms to ensure outcrossing usually have inbreeding depression and elevated genetic loads. Because the flower dichogamy in avocado does not prevent inbreeding, the amount of genetic load in avocado should be less than in other SI species. Gametophytic incompatibility has not been found in avocado. Pollen tube competition in the style has been documented and differences have been reported in the rate of penetration of self- versus nonself-pollen with the nonself-pollen tubes growing faster (Sedgley, 1979). 'Bacon' pollen tube growth may be faster than that of 'Hass', thus favoring outcrossing in 'Hass' and selfing in 'Bacon'. It is interesting to note that for both the Florida and California populations, the A type parent ('Hass' and 'Simmonds') had similar outcrossing rates ($\approx$75%), whereas the B types ('Bacon' and 'Tonnage') had highly skewed outcrossing rates of 45% and 96%, respectively.

Differences were noted in the germination rate between 'Hass' and 'Bacon' seed, 92% versus 84%, and this could be evidence of a higher rate of embryo abortion among selfed fertilized embryos of 'Bacon'. In the Florida study, differences were also noted in germination rates of 'Tonnage' and 'Simmonds' seed, 66% versus 84%, respectively (Borrone et al., 2008). One unusual phenotype was detected in a small number of seedlings of 'Simmonds' (less than 40) in which seeds initiated germination but only produced roots. Unfortunately, these seedlings were not genotyped. They could possibly represent another lethal phenotype resulting from self-pollination. Genetic selection against specific genotypes has been demonstrated in avocado. Using isozyme markers, Degani et al. (1986) demonstrated that selection occurred against fruitlets with the homozygous slow allelic configuration at the leucine amino-peptidase 2 locus. Our observations of lethal mutants in selfed progeny of 'Hass' and 'Bacon' support this observation and we would expect some lethal genotypes to be expressed much earlier in development causing immature fruit to be aborted.

Self-pollination is common in avocado (Davenport, 1986). However, when A and B types are planted together, crosspollination was found to be more prevalent in Florida orchards and among seedlings of 'Hass' in California. The relationship between outcrossing and yields has been much debated and depends on many factors. Results from Bergh et al. (1966) and Arpaia et al. (2005) suggested that pollinizer cultivars in commercial groves increase productivity and this is directly related to the proximity of the commercial cultivar to the pollinizer. Other studies indicated no or little benefit from having pollinizer rows in the orchard (Davenport, 1986; Garner et al., 2008). Using microsatellite markers, Garner et al. (2008) found high outcrossing rates when 'Hass' trees were adjacent to a pollinizer tree. Davenport et al. (2007) also found high outcrossing rates in pollinizer trees and adjacent 'Hass' trees in California commercial orchards. However, as the distance from the pollinizer tree increased, the amount of selfing in 'Hass' trees greatly increased without a concomitant reduction in yield (Ying et al., 2009).

We have assayed 109 microsatellite loci in 'Hass' and 'Bacon' and found that 'Hass' is heterozygous at 61 loci (56%), whereas 'Bacon' is heterozygous at 64 loci (59%) (unpublished data). These percentages are very different from what Alcaraz and Hormaza (2007) found for 16 microsatellite loci, in which 'Hass' was heterozygous for 11 of 16 (69%) and 'Bacon' was heterozygous for 15 of 16 (94%). The values in our study are also lower than those found by Ashworth and Clegg (2003) using 25 microsatellite markers, in which 'Hass' was 72% heterozygous and 'Bacon' was 84% heterozygous. Most of the microsatellite markers used for estimates of heterozygosity in this study were developed from expressed sequence tags (ESTs), not genomic DNA (Borrone et al., 2007). Ten diverse avocado cultivars were surveyed by Borrone et al. (2007) and the observed heterozygosity (H_o) was less than the expected heterozygosity (H_e) for these EST-derived microsatellite markers. We suspect that microsatellite markers derived from ESTs are more conserved than those derived from genomic DNA and this may explain the differences between our estimates of heterozygosity and previously published estimates for 'Hass' and 'Bacon'.

This is the first report of lethal mutants in avocado and two new phenotypes, ''gnarly'' and ''spindly,'' have been described (Figs. 1 and 2). Our study is based solely on data from germinated seedlings and we allowed germination to continue over a period of 12 months in the greenhouse to maximize the number of seedlings we could obtain. We also moved gnarly mutants into a deep shade, whereas the ''spindly'' mutants were maintained in 50% shade. Thus, our discovery rates for mutants may be higher than in avocado breeding programs in which only the most vigorous seedlings are retained. The ''gnarly'' phenotype was only found among seedlings resulting from self-pollination of 'Bacon'. The trait is likely controlled by a single locus in which 'Bacon' is heterozygous and the homozygous recessive produces the ''gnarly'' phenotype. This type of single gene lethal mutant has been reported in *Arabidopsis* and is usually associated with auxin regulation and may involve auxin gradients in various tissues (Hobbie et al., 2000). The ''spindly'' phenotype was only found among seedlings resulting from self-pollination of 'Hass' and is consistent with a two-locus ''dominant negative'' model in which products of independently segregating loci interact to negatively regulate a phenotype (reviewed in Veitia, 2007). In *Arabidopsis*, mutants at a single locus *SPINDLY* (*SPY*), a negative regulator of the GA signal transduction pathway (Jacobsen et al., 1996), exhibit a phenotype similar to that observed in the 'Hass' selfs.

The F_1 progeny from the 'Hass' and 'Bacon' reciprocal crosses, along with selfed seedlings, have been planted in the field at the SHRS in Miami and will be used to develop

a linkage map for avocado. This map is currently under construction using the same techniques as used in the linkage map reported by Borrone et al. (2009).

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