Morphological and RAPD marker evidence of gene flow in open-pollinated populations of *Cucurbita moschata* interplanted with *C. argyrosperma*

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Abstract

The tropical pumpkins *Cucurbita moschata* Duchesne and *C. argyrosperma* Huber are often found growing together. Both *C. argyrosperma* subsp. *argyrosperma* (the domesticated cushaw squash) and the wild species *C. argyrosperma* subsp. *sororia* (L.H. Bailey) Merrick & Bates are found together in Central America. Several reports indicate that plants displaying a phenotype combining traits of both species occur, suggesting gene flow between species. This research studied gene flow between species by evaluating progenies from field plantings of *C. argyrosperma* (both subspecies) interplanted with *C. moschata* and allowed to open-pollinate. The progenies evaluated were from *C. argyrosperma* seed parents. Morphological and species-specific RAPD markers demonstrated that *C. moschata* genes were introgressed into these open-pollinated progeny.

INTRODUCTION

An extensive study by Merrick (1991) of *C. argyrosperma* (including the domesticated subspecies *argyrosperma* and the wild or weedy subspecies *sororia*) concluded that, among the *Cucurbita*, this species is the most closely related to *C. moschata.* Hand pollinations between these species result in fertile progeny when *C. argyrosperma* is used as the seed parent (Merrick 1991; Wessel-Beaver et al. 2004). Both species occupy a similar ecogeographic area of Central American (Whitaker and Davis, 1962). Anecdotal evidence, in the way of reports of intermediate fruit types, suggests that gene flow occurs naturally between these species. Wessel-Beaver (2000) removed all staminate flowers from plants of both *C. argyrosperma* subspecies planted in a field of *C. moschata* and allowed open-pollination. All plants of *C. argyrosperma* produced large numbers of fruit with well-developed seeds. However, the study did not include an evaluation of plants produced from these putative interspecific F_1 seeds.

The objective of this study was to study naturally open-pollinated progeny from *C. argyrosperma* interplanted in fields of *C. moschata* in order to test the hypothesis

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that gene flow occurs from *C. moschata* into *C. argyrosperma* under natural field conditions.

MATERIALS AND METHODS

Field materials and methods

In Isabela, Puerto Rico, four plants of *C. argyrosperma* subsp. *sororia* 'Sor 1(P)' (originating from Panama and obtained from T. Andres) were direct seeded in a 0.25 ha field of *C. moschata* 'PRLongvineSLR'. Six plants of *C. argyrosperma* subsp. *argyrosperma* 'Arg 51-5' (originating from Mexico and obtained from T. Andres) were direct seeded in a different 0.25 ha field of *C. moschata* 'PRLongvineSLR'. Sor 1(P) was planted 15 days, and Arg 51-5 was planted 5 days after PRLongvineSLR to insure flowering at the same time. Fifty-nine open-pollinated fruits were harvested from Sor 1(P) and 8 fruits were harvested from Arg 51-5. As is typical of wild species, *C. sororia* produces many small fruit while the domesticated *C. argyrosperma* produces few fruit per plant. Seeds were extracted from harvested fruits. From open-pollinated Sor 1(P) fruits, 1180 seeds (20 seeds from 59 fruit) were direct-seeded in the field in Isabela. From open-pollinated Arg 51-5, 160 seeds (20 seeds from 8 fruits) were planted. Number of emerged plants was recorded and each plant was evaluated for the set of morphological traits described in Wessel-Beaver et al. (2004) with the purpose of determining if the plant was a putative interspecific F_1. DNA was extracted from all putative F_1 plants as well as a sample of non F_1 plants, as described below. A primer that produced unique RAPD bands in *C. moschata* was used (described below) to determine if a sampled plant (from seeds extracted from the open-pollinated Sor 1(P) or Arg 51-5 fruits) was to be classified as an interspecific F_1.

RAPD protocol

DNA was extracted using the extract solution of Brown and Myers (1998), then following the protocol of Afanador et al. (1993) and Gonzalez et al. (1995), with some modifications. DNA concentrations were determined using a Bio-Rad Smart Spec 3000 spectrometer (Hercules, California). Six-plant bulks of each of the six accessions (two each of *C. moschata* [PRLongvineSLR and PRShortvine1], *C. argyrosperma* [Arg 51-5 and Arg 182-2] and *C. sororia* [Sor 1 (P) and Sor 177-1] were prepared. Bulks were amplified with 40 random decamer primers (Operon Technologies, Inc., Alameda, California). PCR amplification was carried out in a Bio-Rad icycler (Hercules, California) following the protocol of Gwanama et al. (2000). Fragments were separated on a 1.5 % agarose gel stained with ethidium bromide. Band size was determined using the program Quality One (Bio-Rad, Hercules, California). Based on evaluation of the six accessions described above, the primer OP-T01 (5' GGGCCACTCA 3') was identified as the best for identifying interspecific F_1s (Sor 1(P) or Arg 51-5 progeny resulting from pollination by *C. moschata*). This primer produced two clear bands (of 440 and 480 bp size) unique to both accessions of *C. moschata* (data not shown).

RESULTS AND DISCUSSION

Seed harvested from Sor 1(P) open-pollinated fruits

Of the 1180 seeds sampled from open-pollinated Sor 1(P) fruits and planted in the field, only 45 (4 %) germinated. It was noted that seed dormancy was likely at play since plants were still observed to be emerging 60 days post-planting. Of the germinated plants, 73 % were classified as F1's based on the morphological traits described by Wessel-Beaver et al. (2004). Traits considered included fruit width, length and weight, seed width, length and size of margins, the ratio of the peduncle width where it joints with the fruit to the width where it joints with the stem, and rind lignification. Wessel-Beaver et al. (2004) observed that interspecific F_1 plants typically have characteristics that are intermediate between the two parent species. In fact, in this study we did not even individually measure these traits in each plant, but rather could easily identify the interspecific versus Sor 1(P) plants by simple observation (Figure 1).

In the RAPD marker test (using the presence of the two bands produced by primer OP-T01 and unique to *C. moschata* as the criteria) all plants classified in the field as putative F_1s based on morphology were confirmed to be so. The putative F_1s all carried the two markers typical of *C. moschata*, while the plants classified in the field as Sor1(P) did not.

Seed harvested from Arg 51-5 open-pollinated fruits

Of the 180 seeds sampled from open-pollinated Arg 51-5 fruits and planted in the field, more than 90 % germinated. This large difference in germination of the open-pollinated progeny (4 % for progeny from Sor 1(P) versus >90 % for Arg 51-5) likely is related to the fact that the latter species is domesticated while the former is not. Domestication and selection have removed genes controlling seed dormancy that are common in wild species (Bewley 1997; Baskin and Baskin 2004). Again, plants were classified as either interspecific F_1s or as Arg 51-5. Of the emerged plants, 14 % were classified as putative F_1s, a percentage much lower than with seed harvested from Sor 1(P). Since it is likely that many of the seeds from the Sor 1(P) seed parent did not germinate due to dormancy (and thus could not be considered to determine the percentage of F_1 plants), the actual percentage of F_1 progeny was probably far less than the observed 73 %. In contrast to the Sor 1(P) x *C. moschata* F_1s above, the Arg 51-5 x *C. moschata* putative F_1s typically did not have intermediate morphological characteristics. Instead, these plants looked very much like those of the *C. moschata* parent. Wessel-Beaver et al. (2004) observed the same. The form of the peduncle was most helpful in identifying interspecific F_1s. The peduncles of *C. argyrosperma* fruits are very wide and not flared at the base at maturity, while the peduncles of *C. moschata* fruits and the F_1s flare at the base. The interspecific F_1s had peduncles like that of *C. moschata* (Fig. 1).

In the RAPD marker test, all plants classified in the field as putative F_1s were confirmed to be so. The putative F_1s classified as such by morphological inspection all carried the two markers typical of *C. moschata*, while the plants classified in the field as Arg 51-5 did not.

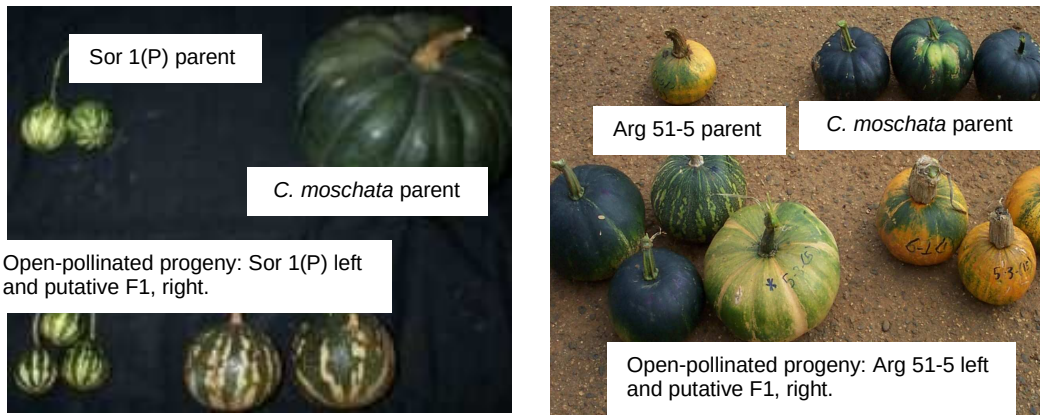

Figure 1. Fruits in lower part of each of the above photographs are open pollinated fruits harvested from plants of either *Cucurbita argyrosperma* ssp. *sororia* (left) or *C. argyrosperma* ssp. *argyrosperma* (right) interplanted in a field of *C. moschata.*

CONCLUSION

Our results suggest that there is probably regular movement of genes from populations of *C. moschata* to populations of *C. argyrosperma* (either domesticated or wild/weedy). In Central America it is likely that fruits pollinated by a sister species are often harvested and intermixed with intraspecific fruits. *C. argyrosperma* and *C. moschata* are also occasionally sympatric in other areas of the world such as the U.S. and China and it is equally likely that some introgression occurs in these places as well. Nevertheless, in Central America both species continue to be quite distinct, perhaps mostly due to human management and selection. *C. argyrosperma* subsp. *argyrosperma* is mostly used for confections or snacks, while *C. moschata* is selected for its attractive flesh. The wild (and probably ancestral) *C. argyrosperma* subsp. *sororia* is found from Mexico south to Panama (Andres T, pers. com.), so a wild-domesticated gene pool complex can only occur in that part of the world. The relative ease with which genes appear to flow between these species should be taken into account when considering the use of transgenic cultivars (although as of this writing, the authors know of no transgenic *C. moschata* or *C. argyrosperma* cultivars).

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