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Inter- and Intracultivar Variation of Heirloom and Open-pollinated Watermelon Cultivars

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Abstract. **Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] cultivars exhibit diverse phenotypic traits, yet are derived from a narrow genetic base. Heirloom cultivars, and to a lesser extent modern open-pollinated (OP) cultivars, are perceived to contain vital genetic variation that is critical for biodiversity conservation and crop improvement. The objective of this study was to characterize the diversity of six heirloom and open-pollinated watermelon cultivars that are popular among U.S. organic, direct-market, and home gardeners. An additional evaluation was conducted to determine whether significant phenotypic and genotypic variation existed among seed lots sourced from different commercial seed vendors. Important horticultural traits such as days to germination, days to first flower, yield, and fruit quality were measured over two field seasons. Genetic diversity was estimated using 32 simple sequence repeat (SSR) markers. Significant differences in horticultural traits among seed lots in both years were observed only in days to germination and first male flower, which may be a consequence of vendor differences in seed storage and quality control. Heirloom 'Moon and Stars' and modern OP 'Sugar Baby' were the most genetically distinct from the other cultivars and heirloom 'Georgia Rattlesnake' was determined to be highly related to the modern OP 'Charleston Gray'. The two heirloom cultivars were observed to have lower average gene diversity than the modern cultivars. Heirloom 'Moon and Stars' contained significant genetic variation among seed lots, yet heirloom 'Georgia Rattlesnake' contained none. These findings suggest that genetic variation can be more accurately attributed to pedigree and foundation seed maintenance practices than to the "heirloom" designation per se. The variation reported in this study can be used to inform conservation and breeding efforts.**

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] is a warm-season annual vegetable crop that is grown on 3.5 million hectares worldwide (Food and Agriculture Organization of the United Nations, 2014). Cultivars express a wide range of phenotypes including fruit size, flesh color, rind pattern, disease resistance, and sweetness.

Despite geographic and phenotypic diversity, the genetic variation of cultivated watermelon is limited (Levi et al., 2001b). Analysis of genome-wide diversity revealed that cultivars from Asia, Europe, and America are derived from one of three subsets of sweet watermelon accessions from Africa (Nimmakayala et al., 2014b). As such, estimates of genotypic variation among cultivars have been low. The genetic diversity among 130 edible-type accessions sampled throughout the world was estimated at 5% (Nimmakayala et al., 2014a). Levi et al. (2001b) found that 46 American cultivars varied by 0.4% to 8%. East Asian and American cultivar types were found to be genetically similar by some analyses (Nimmakayala et al., 2014a; Reddy et al., 2015) but as distinct ecotypes in others (Guo et al., 2013; Zhang et al., 2012). The resequencing of 20 watermelon accessions shows that watermelon is less genetically diverse than maize, soybean, and rice (Guo et al., 2013). In all, these findings are consistent with a severe genetic bottleneck during domestication.

Conservation of genetic variation is critical to crop improvement through plant breeding. Farmer-maintained landraces are favorable sources of genetic variation because they are more adapted to agricultural production than wild relatives (Villa et al., 2005). By their nature, open-pollinated (OP) cultivars maintain greater population-level genetic diversity than hybrid seed types, which are derived from the cross-pollination of two inbred parental lines. A benefit to the grower is that seed of OP cultivars can be saved from year to year, unlike hybrid seed that does not grow true-to-type in subsequent generations and thus must be purchased from seed companies each season. Due to these realized and perceived benefits, organic, direct-market, and home growers have inspired a renewed interest in OP cultivars (Phillips, 2016).

Today, farmer-maintained landraces in industrialized countries are rare (Thomas et al., 2011). In the United States, the designation "heirloom" is considered by some as analogous to landrace in that heirlooms are perceived to be locally adapted and genetically diverse. In this study and in present-day seed catalogues, "heirloom" is defined as a cultivar that was introduced before the advent of modern breeding techniques (the year 1942 is a commonly used temporal threshold) by farmers or nonprofessional breeders (DeMuth, 1998). However, the development of the modern seed industry has made the term ambiguous. If one considers that commercially distributed heirloom varieties are maintained and multiplied in a similar fashion to modern OP cultivars within a "certified seed" model (Parlevliet, 2007), rather than maintained through ongoing recurrent selection by end users, then the public perception of heirloom cultivars as more diverse than modern OP cultivars is questionable.

As a related but separate issue, the discovery of within-cultivar variation, whether in heirloom or modern cultivars, is of practical relevance to the seed industry and scientific community. Within-cultivar variation is an essential genetic resource in the maintenance and improvement of elite cultivars in a changing climate (Berry et al., 2014). Numerous studies report that significant variation of many agronomic traits were observed within inbred S_5 to S_{20}+ lines from different seed stock sources (reviewed in Tokatlidis, 2015), which is invaluable information to breeders. In these cases, cultivars and inbred lines assumed to be pure lines undergo changes when they are regenerated and/or maintained in separate locations; when properly characterized, this variation can be used for cultivar improvement and the conservation breeding of elite cultivars.

The OP cultivars featured in this study are not covered by plant variety protection (PVP) and thus foundation seed maintenance is unregulated and likely decentralized (M. Colley, personal communication). The term "foundation seed" in the present study refers to seed stock from which commercial seed is multiplied, but does not imply the formal designation associated with state-certified seed programs. It is expected that seed multiplied from independent foundation seed stocks, particularly in an unregulated model, is more likely to be genetically differentiated than certified seed covered by PVP. Significant variation among seed lots of cultivars sourced from different seed companies should be considered in conservation and breeding efforts. Candole et al. (2012) identified differentiated levels of disease resistance among seed lots from different seed companies in the heirloom pepper 'California Wonder', which had long been used as a standard in pathology experiments. This finding helped

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scientists select a more reliable cultivar against which to judge other cultivars in disease screens. A seed lot with greater genetic diversity or with novel alleles may prove more useful in breeding programs than genetically uniform seed lots.

What role, then, does commercial seed production play in the conservation of genetic diversity of heirloom and modern OP cultivars? A balance between the maintenance of cultivar integrity and the conservation of genetic diversity must be sustained.

Cultivars must satisfy distinctness, uniformity, and stability (DUS) standards (Union for the Protection of New Varieties of Plants, 2002) and are perceived to be uniform genotypes. Consequently, in commercial seed production, emphasis is placed on rogueing off-type and diseased plants to maintain uniform and high-quality seed (Parlevliet, 2007). Guidelines for isolation distance and minimum population size during multiplication and maintenance of foundation seed vary by crop (George, 2013). These practices safeguard against genetic drift and gene flow between cultivars. The extent to which cultivar purity strategies, during both foundation seed maintenance and multiplication, are practiced by each commercial seed company is unknown.

By a somewhat competing natural phenomenon, cultivars are *not* genetically uniform due to abundant biological mechanisms that ensure adaptability of genomes. Genetic variation is inherent to cultivars via natural processes, including 1) heterogeneity in the progenitor gene pool, 2) de novo mutation, 3) genetic drift, and 4) environmentally triggered alterations to the genome. Artificial forces also affect intracultivar diversity during commercial seed propagation, including 1) unintentional gene flow during seed propagation, 2) bottlenecks during establishment of foundation or multiplication seed stocks, and 3) unintentional selection as a result of environmental conditions or management practices. Although most genetic variation is derived from the progenitor gene pool, de novo variation has been estimated as high as 18% of total variation in single-plant derived soybean lines (Yates et al., 2012).

Genetic variation is not necessarily a condition to be avoided, but in fact is an essential mechanism to exploit for long-term maintenance of cultivars and in breeding improved cultivars. Useful intracultivar genetic variation has been documented in maize (Gethi et al., 2002), soybean (Yates et al., 2012), rice (Olufowote et al., 1997), cotton (Hinze et al., 2012), sunflower (Zhang et al., 1995), olive (Caruso et al., 2014), and mango (Singh et al., 2009), and the selection of superior lines has been used to improve performance or quality of the cultivars. Therefore, although unadapted germplasm is often regarded as the prime source of novel alleles for crop improvement, there is actually a great deal of incremental progress that can be made when plant breeders exploit the variation within elite cultivars (Rasmusson and Phillips, 1997).

The forces described above that drive intracultivar variation should be explicitly addressed during the production of breeder and foundation seed for long-term cultivar maintenance. Tokatlidis (2015) recommends that breeder seed be maintained through ultra low-density plantings with periodic intense selections and consecutive mild selection. The intense selection period requires the selection of top performing sister lines to be evaluated by progeny testing. Wide plant spacing ensures that competition is minimized and that the true genotypic character of each individual is expressed, so that effective evaluation can take place. Beyond the typical rogueing for off-types and diseased seed that takes place in commercial seed propagation, this method ensures that genetic degradation is avoided, high-quality and uniform stocks are maintained, the cultivar can be incrementally improved to meet the demands of long-term environmental changes, and interesting selections can be funneled into alternative breeding pipelines. Small- to medium-scale seed companies that serve organic, direct-market, and home growers may be limited in their ability to follow these conservation breeding recommendations; nonetheless, not explicitly addressing foundation seed maintenance may directly conflict with their customers' desire to grow genetically diverse and adapted cultivars.

The objective of this study was to characterize the diversity of heirloom and modern OP watermelon cultivars popular among U.S. organic, direct-market, and home growers. Phenotypic variation, genetic differentiation, and within-cultivar gene diversity were measured. For each cultivar, variation among seed lots sourced from various commercial vendors was also investigated. Important horticultural traits, such as days to germination, days to first flower, yield, and fruit quality were measured over two field seasons. Despite the low genetic diversity among watermelon cultivars worldwide, the American cultivars that are the focus of this study have been successfully differentiated using a variety of marker systems (Levi et al., 2001b, 2009; Yang et al., 2016). The current study used 32 SSR markers to estimate genetic diversity. Because SSRs have a high mutation rate and are multiallelic, they are an ideal marker choice for studying highly related populations. SSRs have been shown to be more informative than single nucleotide polymorphisms (SNPs) in their ability to detect rare genotypes and to discern genetic distance over a short time span (Hamblin et al., 2007), although as the cost of SNPs per locus continues to decrease, this advantage diminishes. This study is the first attempt to detect intracultivar variation in watermelon and aims to characterize the level of genetic diversity maintained at the commercial seed level to inform conservation efforts. Plant breeders may also benefit from exploiting divergent seed lots for cultivar improvement. A direct comparison of heirloom vs. modern OP diversity serves to clarify the role of both cultivar types in the promotion of biodiversity.

Materials and Methods

Plant material. Seeds of six heirloom and modern OP cultivars were obtained from various commercial seed vendors for a total of 24 seed lots (Table 1). Inquiries were made to the seed vendors to gather information on seed lot origin and multiplication methods, but companies were either limited or unwilling to provide such details due to proprietary claims or traceability constraints. The following pedigrees were obtained from Wehner and Mou (2013). Sugar Baby is a modern OP cultivar developed by M. Hardin in 1955 by inbreeding a selection of Tough Sweets for 13 years. Crimson Sweet is a modern OP cultivar developed by Charles V. Hall at Kansas State University in 1963 using pedigree selection of ('Miles' × 'Peacock') × 'Charleston Gray'. Moon and Stars is an heirloom cultivar with unknown parentage, developed by an unknown farmer in Colorado and released by Peter Henderson and Company in 1926. Charleston Gray is a modern OP cultivar developed by the Southeastern Vegetable Breeding Laboratory in 1954 as a pedigree selection of {[('Africa 8' × 'Iowa Belle') × 'Garrison'] × 'Garrison'} × [('Hawkesbury' × 'Leesburg') × 'Garrison']. Georgia Rattlesnake is an heirloom cultivar developed by M.W. Johnson in 1870 using unknown parentage. Congo is a modern OP cultivar bred by the Southeastern Vegetable Breeding Laboratory in 1949 as a pedigree selection of ('African' × 'Iowa Belle') × 'Garrison'. Seeds from each seed lot were sown in the greenhouse on 26 Mar. 2014 and 27 Mar. 2015, into two seedling trays each with Fafard 3B potting mix (Conrad Fafard, Inc., Agawam, MA) in a completely randomized design. Seedlings were transplanted to polyethylene-covered beds at the Durham Horticultural Farm in Watkinsville, GA on 23 Apr. 2014 and 8 May 2015. Each seed lot was randomly assigned to a 10-plant plot in a randomized complete block design with 4 replications. Seedlings were transplanted 1.2 m apart in-row and 1.8 m apart between rows. Fertilizer was applied to plots at the rate of 67 kg·ha^{-1} of N as preplant granular fertilizer (10N–0.9P–6.6K) and 12 kg·ha^{-1} of N as soluble fertilizer (15N–0P–12.5K) applied weekly via drip irrigation. Plants were irrigated 3 times per week as needed to accumulate ≈2.5 cm water per week. Leaf samples were collected in the field from the third, fifth, and seventh plant per plot, immediately frozen in liquid nitrogen, and stored at –80 °C until further processing.

Horticultural traits. Days to germination was recorded in the greenhouse for each seed based on the criteria of full cotyledon expansion. Days to first male and female flower were recorded for each plant in the field from 1 May to 15 June in 2014 and from 15 May to 30 June in 2015. Marketable fruit from each plot were harvested, weighed, and counted on 27 June and 7 July in 2014 and on 17 July and

Table 1. Heirloom (H) and modern open-pollinated (OP) watermelon cultivars and seed vendors used for phenotypic and genotypic analysis.

Cultivar	Type	Seed lot	Vendor	Germination (%) 2014	2015
Sugar Baby	OP	1	Johnny's Selected Seeds[z]	89	93
		2	Clifton Seed Company[y]	94	94
		3	High Mowing Organic Seeds[x]	96	98
		4	Harris Seeds[w]	93	95
		5	NE Seed[v]	93	98
Crimson Sweet	OP	6	Baker Creek Heirloom Seeds[u]	90	91
		7	Johnny's Selected Seeds	89	98
		8	Clifton Seed Company	86	88
		9	High Mowing Organic Seeds	83	87
		10	Harris Seeds	90	97
Moon and Stars	H	11	Seed Savers Exchange[t]	85	90
		12	NE Seed	81	83
		13	High Mowing Organic Seeds	89	96
		14	Sow True Seed[s]	90	62
		15	Sustainable Seed Company[r]	68	71
Charleston Gray	OP	16	Baker Creek Heirloom Seeds	83	95
		17	NE Seed	93	82
		18	Sow True Seed	89	88
Georgia Rattlesnake	H	19	Baker Creek Heirloom Seeds	92	90
		20	Clifton Seed Company	85	85
		21	Sow True Seed	89	84
Congo	OP	22	Baker Creek Heirloom Seeds	72	71
		23	Clifton Seed Company	74	65
		24	Sustainable Seed Company	88	89

[z]Johnny's Selected Seeds, Winslow, ME.
[y]Clifton Seed Company, Faison, NC.
[x]High Mowing Organic Seeds, Walcott, VT.
[w]Harris Seeds, Rochester, NY.
[v]NE Seed, East Hartford, CT.
[u]Baker Creek Heirloom Seeds, Mansfield, MO.
[t]Seed Savers Exchange, Decorah, IA.
[s]Sow True Seed, Asheville, NC.
[r]Sustainable Seed Company, Chico, CA.

21 July in 2015. Two representative fruit from each plot were weighed individually and cut to measure fruit length, width, and rind thickness. Firmness of flesh was measured at 2 locations per fruit, off-centered in the endocarp heart, using a handheld penetrometer with 10 mm solid probe (Certified Material Testing Products, Palm Bay, FL). Soluble solids content was measured for 1 teaspoon of watermelon juice using a handheld refractometer (Spectrum Technologies, Plainfield, IL). Analysis of variance was conducted using Stata version 13 (StataCorp, College Station, TX) and means were separated using Fisher's protected least significant difference at $P \leq 0.05$.

DNA extraction and SSR analysis. Twelve individuals per seed lot were genotyped. Frozen leaf samples collected in 1.5-mL microtubes were ground using 5-mm steel beads in a TissueLyser (Qiagen, Inc., Valencia, CA) for 30 s then DNA was extracted using the E-Z 96 Plant DNA Kit (Omega Bio-Tek, Norcross, GA). Extracted DNA was quantified using the Tecan Infinite M200 Pro (Tecan, Morrisville, NC) and diluted to 20 ng·μL^{-1}. Thirty-eight SSR loci reported as variable among commercial cultivars (Joobeur et al., 2006; Ren et al., 2012; Zhang et al., 2012) were tested, and 32 polymorphic loci that were evenly distributed among watermelon chromosomes were selected (Table 2) for genetic analysis. Polymerase chain reaction (PCR) was conducted using the M13 universal primer system (Schuelke, 2000) in which the M13 sequence (5′-TGTAAAACGACGGCCAGT-3′) was appended to the 5′ end of the forward primer sequence, the reverse primer sequence was unaltered, and an M13 primer labeled with FAM, TAM, or HEX fluorescent dye was added to each reaction. PCR was conducted separately for each locus with reactions containing 20 ng DNA template, 1× standard Taq buffer (New England Biolabs, Ipswich, MA), 0.1 mM dNTP (Qiagen, Inc.), 0.1 μM M13-appended forward primer, 0.4 μM reverse primer, 0.4 μM dye-labeled M13 primer, 0.6 U Taq DNA polymerase (New England Biolabs) in a 20 μL total volume. PCR was conducted using MyCycler (Bio-Rad, Hercules, CA) with the following program: 90 s initial denaturation at 95 °C; 10 cycles of 15 s denaturation at 95 °C, 20 s annealing at 53 °C (–1 °C each subsequent cycle), and 30 s of extension at 72 °C; 35 cycles of 15 s denaturation at 95 °C, 20 s annealing at 43 °C and 30 s of extension at 72 °C; and 15 min final extension at 72 °C. PCR products were diluted 2 to 4x depending on agarose band density, pooled into sets with each of the 3 unique fluorescent dyes present, and added to formamide with a GeneScan-500 ROX internal-lane size standard (ABI; Applied Biosystems by Life Technologies Corporation, Carlsbad, CA). Product fragment lengths were measured on the Applied Biosystems 3730xl 96-capillary DNA Analyzer (ABI) at the Georgia Genomics Facility (Athens, GA).

Alleles were interpreted from fluorescent peaks using Geneious version R8.1 (Kearse et al., 2012). Power analysis was conducted by estimating genetic diversity (mean expected heterozygosity) vs. number of loci via 1000 random permutations of the data using MultiLocus version 1.3b (Agapow and Burt, 2001). Genetic parameters were analyzed using GenAlEx version 6.502 (Peakall and Smouse, 2012). Confidence intervals for gene diversity were obtained by 1000 bootstraps using the PopGenKit package (Paquette, 2012) in R (R Core Team, 2017). Polymorphism information content (PIC) was calculated using the formula PIC = $1 - \Sigma P_{ij}^2$, where P_{ij} is the frequency of j^{th} allele of the i^{th} locus (Botstein et al., 1980). Analysis of molecular variance (AMOVA) and F-statistics were calculated for codominant allelic distance in a $2N \times 2N$ matrix and tested using 999 standard permutations and significance threshold of $P \leq 0.05$ (Michalakis and Excoffier, 1996). Nei's standard genetic distance (Nei et al., 1983) was used to construct a principal coordinate analysis (PCoA) in GenAlEx and infer an unrooted neighbor-joining (uNJ) tree (Saitou and Nei, 1987) using MEGA version 4 (Tamura et al., 2007).

Results

Horticultural traits. Intercultivar variation was apparent for all horticultural traits as expected (Table 3). Analysis was conducted separately for each year due to a by-year interaction. Variation among seed lots of the same cultivar was detected for some traits as follows. Days to germination differed among seed lots of 'Sugar Baby' in 2014; 'Georgia Rattlesnake' in 2015; and 'Crimson Sweet', 'Moon and Stars', and 'Congo' in a consistent pattern both years (Table 4). Days to first male flower differed among seed lots of 'Georgia Rattlesnake' in 2014; 'Moon and Stars' in 2015; and 'Crimson Sweet', 'Charleston Gray', and 'Congo' both years.

Intracultivar variation was detected for days to first female flower in 'Sugar Baby' and 'Georgia Rattlesnake' in 2014 only (Table 4). 'Sugar Baby' seed lots varied in yield and fruit count in 2014 but not 2015. Fruit count differed among 'Crimson Sweet' seed lots in 2014 only. Variation in rind thickness was found among 'Congo' seed lots in 2015. Finally, soluble solids content, a proxy for sweetness, differed among 'Moon and Stars' and 'Charleston Gray' seed lots in 2014. There was no significant difference among seed lots for fruit weight, length, width or flesh firmness (Supplemental Table 1) in either year.

Because flowering time may be a consequence of days to germination, Pearson's correlation was used to assess the relationship between the traits. In 2014, there was a strong correlation between days to germination and days to first male flower ($r = 0.548$, $P < 0.001$). The correlation between days to first female flower and days to germination was moderate ($r = 0.334$, $P = 0.001$). In 2015, the correlation between days to germination

Table 2. Simple sequence repeat primers and genotypic parameters of seed lots from various commercial seed vendors for six heirloom and open-pollinated watermelon cultivars.

Locus	Reference	Chr.	Start location[z]	Stop location[z]	Fragment size range (bp)	Na	PIC[y]
BVWS00048	Zhang et al. (2012)	3	27,914,506	27,914,682	177–179	2	0.44
BVWS00067	Ren et al. (2012)	11	1,947,675	1,947,549	140–152	3	0.15
BVWS00102	Ren et al. (2012)	4	15,377,126	15,376,994	148–152	2	0.06
BVWS00106	Zhang et al. (2012)	5	29,257,732	29,257,588	156–212	4	0.36
BVWS00155	Zhang et al. (2012)	1	30,213,324	30,213,162	171–185	6	0.54
BVWS00177	Ren et al. (2012)	9	4,023,989	4,024,182	180–202	4	0.74
BVWS00208	Zhang et al. (2012)	4	1,8760,155	18,760,327	150–190	5	0.64
BVWS00209	Zhang et al. (2012)	9	34,063,455	34,063,581	122–136	4	0.36
BVWS00215	Ren et al. (2012)	5	3,566,759	3,566,639	134–140	2	0.05
BVWS00225	Ren et al. (2012)	7	2,785,791	2,785,950	178–184	2	0.34
BVWS00228	Zhang et al. (2012)	11	23,231,725	23,231,570	156–202	7	0.54
BVWS00233	Zhang et al. (2012)	6	23,367,900	23,367,738	172–180	2	0.34
BVWS00236	Zhang et al. (2012)	10	1,007,583	1,007,748	187–194	2	0.35
BVWS00244	Ren et al. (2012)	3	3,416,523	3,416,669	159–165	2	0.21
BVWS00287	Ren et al. (2012)	6	17,381,037	17,381,191	169–173	2	0.00
BVWS00297	Zhang et al. (2012)	2	34,241,669	34,241,531	150–166	6	0.64
BVWS00314	Zhang et al. (2012)	2	23,078,022	23,078,158	148–154	2	0.09
BVWS00333	Zhang et al. (2012)	9	21,608,401	21,608,274	132–144	3	0.64
BVWS00433	Zhang et al. (2012)	7	4,151,409	4,151,009	269–296	3	0.57
BVWS00441	Zhang et al. (2012)	5	12,518,305	12,518,477	178–196	3	0.45
BVWS00522	Ren et al. (2012)	8	8,496,014	8,495,740	279–289	2	0.14
BVWS00839	Zhang et al. (2012)	11	10,534,583	10,534,832	248–268	4	0.53
BVWS00948	Zhang et al. (2012)	1	22,668,108	22,668,377	278–286	2	0.49
BVWS01001	Ren et al. (2012)	8	11,630,160	11,629,919	259–263	2	0.49
BVWS01199	Ren et al. (2012)	3	12,292,958	12,293,157	217–226	3	0.02
BVWS01836	Ren et al. (2012)	1	1,185,151	1,185,437	291–316	3	0.01
BVWS01911	Ren et al. (2012)	2	16,239,566	16,239,832	262–276	3	0.06
BVWS02048	Zhang et al. (2012)	10	15,916,596	15,916,844	256–278	3	0.32
BVWS02205	Ren et al. (2012)	10	26,080,606	26,080,721	152–186	6	0.65
BVWS02428	Ren et al. (2012)	4	7,356,764	7,356,879	130–134	2	0.50
BVWS02453	Ren et al. (2012)	7	25,558,609	25,558,908	229–316	3	0.09
MCPI-5	Joobeur et al. (2006)	6	26,786,254	26,786,441	208–230	5	0.68
Mean						***3.3***	***0.36***
SE						*0.26*	*0.06*

[z]Chromosome, start, location and stop location based on Guo et al. (2013) genome sequence, reported in bps (bp).
[y]Polymorphism information content (PIC) is $1 - \sum P_{ij}^2$, where P_{ij} is the frequency of j^{th} allele of the i^{th} locus (Botstein et al., 1980).
bp = base pairs; Chr = chromosome; Na = number of alleles.

Table 3. Mean phenotypic values of horticultural traits calculated across multiple seed lots for six heirloom and open-pollinated watermelon cultivars in 2014 and 2015. Values within columns with a different lowercase letter have means that are significantly different at $P \leq 0.05$.

Cultivar	Days to germination	Days to first male flower	Days to first female flower	Yield (1000 kg·ha^{-1})	Fruit count (1000 fruit/ha)	Fruit wt (kg)	Fruit length (cm)	Fruit width (cm)	Rind thickness (cm)	SSC (°BRIX)	Flesh firmness (kg·cm^{-2})
2014											
Sugar Baby	7.3 bc	43.6 a	52.4 a	33.0 c	10.4 a	3.2 c	21.0 d	19.5 c	1.7 b	9.3 b	1.7 a
Crimson Sweet	7.4 bc	46.8 b	60.1 b	42.0 ab	5.9 bc	7.2 ab	27.1 c	24.2 a	1.4 a	10.7 a	1.7 a
Moon and Stars	7.5 c	50.5 d	63.4 c	42.2 a	6.5 b	6.4 b	26.5 c	23.7 a	1.4 a	8.6 c	2.0 b
Charleston Gray	6.7 a	48.2 c	59.5 b	35.3 abc	4.9 cd	7.3 ab	39.7 a	19.8 c	1.5 a	9.8 b	2.0 b
Georgia Rattlesnake	7.0 ab	48.6 c	62.4 c	34.9 bc	5.7 bcd	6.8 ab	39.4 a	19.9 c	1.4 a	9.2 bc	1.9 ab
Congo	10.8 d	56.0 e	65.3 d	33.7 c	4.4 d	7.5 a	36.1 b	20.9 b	1.5 ab	9.0 bc	2.0 b
2015											
Sugar Baby	9.4 a	54.5 a	61.0 a	28.4 c	7.2 a	4.7 c	21.3 e	20.1 d	1.0 a	9.4 c	1.7 b
Crimson Sweet	9.6 a	62.0 b	69.1 c	43.0 b	5.5 bc	9.5 b	28.0 d	25.2 b	1.5 cd	11.5 a	1.2 a
Moon and Stars	9.8 a	61.6 b	69.4 c	56.6 a	5.8 bc	11.8 a	29.6 c	26.8 a	1.6 d	9.4 c	1.2 a
Charleston Gray	10.5 b	61.6 b	68.2 b	47.1 ab	5.1 cd	10.4 b	41.8 a	21.2 c	1.2 b	10.2 b	1.1 a
Georgia Rattlesnake	11.3 c	63.1 c	69.6 c	42.8 b	4.3 d	10.5 b	41.8 a	21.2 c	1.3 bc	10.6 b	1.2 a
Congo	13.1 d	65.1 d	69.8 c	49.1 ab	6.5 ab	10.0 b	38.4 b	21.9 c	1.3 b	9.6 c	1.5 b

SSC = soluble solid content.

and first male flower was moderate (r = 0.443, P = 0.000), but the correlation with days to first female flower was not significant. Because days to germination may be influenced by an assortment of environmental factors, such as seed storage conditions and age of seed, rather than genetic variation alone, it is possible that the intracultivar variation detected in days to germination and flowering time are a consequence of the vendors' seed quality practices. Although quality control is an important component of commercial seed production, it is beyond the scope of our study.

Overall, the presence of phenotypic variation for horticultural traits among seed lots of a particular cultivar was limited, and the detection of variation consistently both years was rarer still; this indicates that for most horticultural traits, there is not strong evidence of phenotypic divergence among seed lots during commercial seed production. Seed companies are responsible for enforcing maintenance selection during seed multiplication (Parlevliet, 2007). Traits such as fruit size, rind pattern, and sugar content are relatively easy to maintain through rogueing off-types. Traits such as yield and disease resistance are harder to maintain due to genotype × environment interactions, in which such traits may not be observed unless a particular environmental condition is present. Also, divergence of seed lots for environmentally

Table 4. Mean phenotypic values of horticultural traits of seed lots from various commercial seed vendors for six heirloom and open-pollinated watermelon cultivars. Values within cultivars and columns with a different lowercase letter have means that are significantly different at $P \leq 0.05$.

Cultivar	Seed lot	Days to germination		Days to first male flower		Days to first female flower		Rind thickness (cm)		SSC[z] (°BRIX)		Yield (1000 kg·ha^{-1})		Fruit count (10 00 fruit/ha[u])	
		2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Sugar Baby	1	7.5 b	9.3	42.8	54.7	51.2 a	60.6	1.7	0.8	9.4	10	36.5 ab	24.7	12.5 a	6.2
	2	7.3 ab	9.4	43.8	54.4	52.8 ab	60.6	1.8	1.0	9.8	9.2	39.9 a	36.0	11.6 a	8.9
	3	7.1 ab	9.6	44.1	55.1	53.7 b	62.0	1.9	1.0	8.8	9.7	28.7 c	26.4	9.7 b	6.2
	4	7.9 b	9.4	42.3	53.6	50.8 a	60.3	1.4	1.0	9.3	9.0	31.7 bc	28.3	9.7 b	7.5
	5	6.6 a	9.2	45.0	54.7	53.8 b	61.1	1.8	1.0	9.4	9.4	28.0 c	26.7	8.3 b	7.2
Crimson Sweet	6	7.0 ab	9.3 b	46.8 ab	61.0 ab	59.9	68.8	1.4	1.5	11.0	11.4	40.2	47.4	5.9 ab	6.2
	7	6.9 ab	8.0 a	46.5 ab	59.6 a	58.8	68.3	1.6	1.5	11.1	11.3	45.5	43.2	6.6 a	5.5
	8	7.1 b	9.9 b	47.3 b	62.5 c	59.9	69.0	1.3	1.6	10.1	11.4	40.5	39.4	5.6 ab	5.2
	9	9.7 c	12.6 c	48.8 b	64.6 d	61.1	69.0	1.6	1.4	10.3	11.8	39.3	46.0	4.5 b	5.8
	10	6.4 a	8.5 a	44.8 a	62.4 bc	61.3	68.4	1.4	1.5	11.1	11.5	44.4	39.1	6.8 a	4.8
Moon and Stars	11	6.9 a	8.6 a	49.0	60.7 a	63.3	69.5	1.3	1.6	8.5 b	9.6	52.0	65.6	7.5	6.3
	12	8.1 b	11.4 c	51.0	61.2 a	64.3	68.4	1.5	1.6	8.6 b	9.7	44.9	63.1	6.4	6.4
	13	6.9 a	9.6 b	50.2	64.6 b	61.3	68.4	1.2	1.6	8.6 ab	9.5	38.1	48.9	6.7	5.1
	14	7.8 b	9.5 b	51.9	60.0 a	64.1	67.7	1.3	1.7	8.1 b	9.0	36.3	60.1	5.9	6.3
	15	7.7 b	9.8 b	50.4	61.2 a	64.5	69.7	1.5	1.6	9.1 a	9.4	39.9	45.4	6.1	5.0
Charleston Gray	16	6.7	10.2	46.4 a	61.3 a	59.7	67.9	1.5	1.3	8.3 b	10.4	34.2	51.9	5.3	5.7
	17	6.6	10.6	50.0 b	63.0 b	59.1	68.1	1.4	1.1	10.9 a	9.9	35.6	44.4	4.5	4.8
	18	6.8	10.6	48.1 ab	60.3 a	59.6	67.3	1.5	1.3	10.2 ab	10.2	36.0	44.9	5.0	4.8
Georgia Rattlesnake	19	7.0	12.4 c	50.1 b	63.6	64.8 b	69.6	1.2	1.3	8.8	10.6	29.2	44.7	6.6	4.8
	20	7.3	11.5 b	47.5 a	63.8	62.4 a	68.8	1.5	1.2	8.9	10.7	37.9	28.9	5.0	2.9
	21	6.9	9.7 a	48.1 ab	61.9	60.1 a	68.8	1.4	1.5	9.9	10.6	37.5	54.9	5.6	5.2
Congo	22	13.0 c	15.0 b	56.4 b	64.2 a	65.0	70.1	1.4	1.3 ab	9.4	9.2	35.4	45.9	4.6	6.3
	23	11.4 b	14.6 b	54.5 ab	64.4 a	67.1	69.6	1.7	1.5 b	9.6	10.2	24.0	56.2	3.4	7.1
	24	8.6 a	10.4 a	52.6 a	66.5 b	63.9	70.3	1.5	1.1 a	8.2	9.3	41.7	45.0	5.3	6.0

[z]Soluble Solid Content.

influenced traits may occur when multiplication is performed in disparate growing conditions that significantly differ in selective pressure. The limited occurrence of significant intracultivar variation of horticultural traits in the present study indicates that companies are sufficiently maintaining cultivar DUS.

Genetic parameters of the SSR loci. A total of 104 putative alleles across 32 SSR polymorphic loci were detected across the 24 seed lots studied (Table 2). The average alleles per locus of 3.3, with a range of 2 to 7 alleles per locus, is lower than typical diversity studies but not unexpected given the narrow genetic diversity of watermelon cultivars (Levi et al., 2001b). The average diversity (PIC) per locus was 36% (Table 2), which indicates low frequency of minor alleles. Of the 32 loci examined, 10 were very diverse (PIC > 0.50), 11 were minimally diverse (PIC < 0.25), and the remaining 11 were intermediately diverse. Locus BVWS00287 was the least diverse (PIC = 0.003) and locus BVWS00177 was the most diverse (PIC = 0.744). The 32 selected loci demonstrate a wide range in allele number and PIC. Taken together, these genetic parameters reflect the known features of U.S. watermelon cultivars: low genetic diversity and high homozygosity (Levi et al., 2001a). A neighbor joining (NJ) tree constructed from Nei's standard distance (Fig. 1) shows that all seed lots were appropriately assigned into cultivar groups and is consistent with previous studies that used other marker systems (Huayu et al., 2016; Levi et al., 2004, 2009; Yang et al., 2016; Zhang et al., 2012). The tree configuration is further supported by available pedigree information, which includes shared parentage of 'Congo', 'Crimson Sweet', and 'Charleston Gray' and no shared parentage of 'Sugar Baby'. These findings are consistent with the Reddy et al., (2015) study, which found that 'Crimson Sweet' and 'Georgia Rattlesnake' clustered together in a group of African ancestry, 'Congo' and 'Moon and Stars' clustered together in a group of European and American ancestry, and 'Sugar Baby' was placed in a second, genetically distinct group of European and American ancestry; as well as additional studies that describe 'Sugar Baby' as the least related among commercial cultivars (Reddy et al., 2015; Yang et al., 2016).

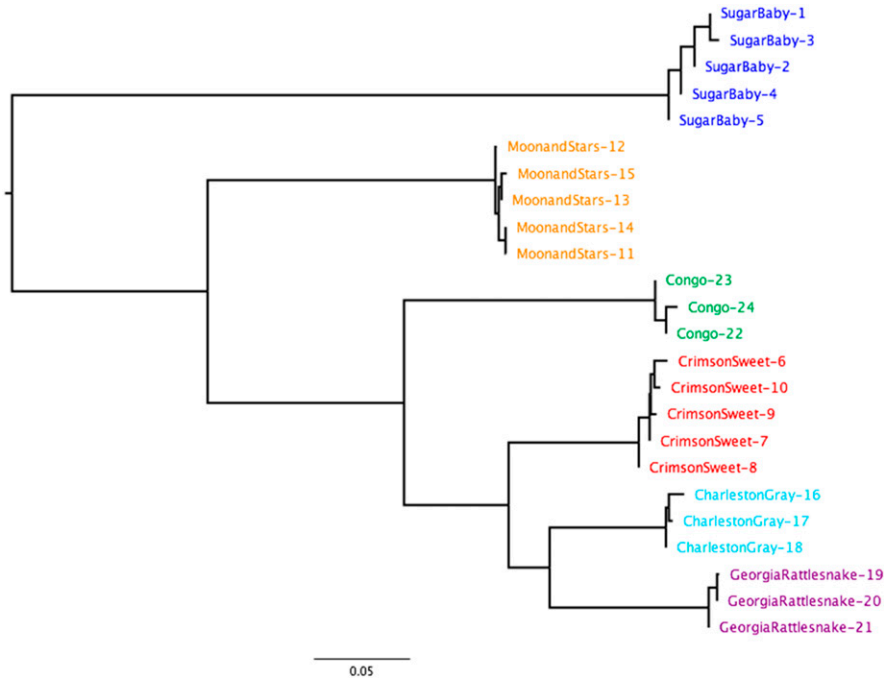

Fig. 1. Neighbor-joining tree using Nei's standard distance (Nei, 1972) for seed lots from various commercial seed vendors for six heirloom and modern open-pollinated watermelon cultivars. The optimal tree with the sum of branch length = 1.12 is shown. The tree is drawn to scale.

Interestingly, the parentage of 'Georgia Rattlesnake' and its use in the pedigree of modern cultivars is undocumented in published cultivar descriptions (Wehner, 2002). The genetic distance estimated in this study (Fig. 1) revealed that it is closely related to 'Charleston Gray' and may be a progenitor parent. This result prompted a thorough review of historical literature, which uncovered a biographical account of Ruben F. Kolb using 'Georgia Rattlesnake' to develop 'Kolb

Gem' in 1885 (Rogers, 1958). 'Kolb Gem' is a documented progenitor of 'Charleston Gray', thus its relatedness to 'Georgia Rattlesnake' is now confirmed.

A post hoc power analysis was conducted to determine the optimal number of loci necessary to estimate genetic diversity using 1000 random permutations of the data (Supplemental Fig. 1). For 'Sugar Baby', 'Charleston Gray', and 'Congo', 95% of genetic diversity can be explained by 22 to 23 loci and the genetic information gained upon adding additional loci begins to plateau. A similar plateau pattern occurs in 'Crimson Sweet', 'Moon and Stars', and 'Georgia Rattlesnake' at 26 to 27 loci. From this analysis, it can be inferred that additional loci beyond the 32 selected for this study would have provided diminishing returns in information gained. Together, the NJ tree and post hoc power analysis suggest that the 32 loci selected for the present study provided sufficient power to address the research objectives.

Genetic diversity among cultivars. Intercultivar diversity was detected for all genetic parameters (Table 5). 'Sugar Baby' was polymorphic at the most loci (%P = 75.0) and contained the greatest average number of alleles per locus (Na = 1.91). 'Georgia Rattlesnake' was polymorphic for the fewest loci (%P = 43.8) and had the least average number of alleles per locus (Na = 1.50). 'Crimson Sweet', 'Moon and Stars', 'Charleston Gray', and 'Congo' were each polymorphic for 59.4% of loci, although only 50% of loci were polymorphic for all four cultivars.

Table 5. Genetic diversity parameters of six heirloom and open-pollinated watermelon cultivars based on 32 simple sequence repeat loci.

Cultivar	%P[z]	Na[y]
Sugar Baby	75.0	1.91
Crimson Sweet	59.4	1.72
Moon and Stars	59.4	1.72
Charleston Gray	59.4	1.69
Georgia Rattlesnake	43.8	1.50
Congo	59.4	1.72
Mean	59.4	1.71
SE	4.0	0.05

[z]Percent polymorphic loci (%P).
[y]Number of alleles, averaged over loci (Na).

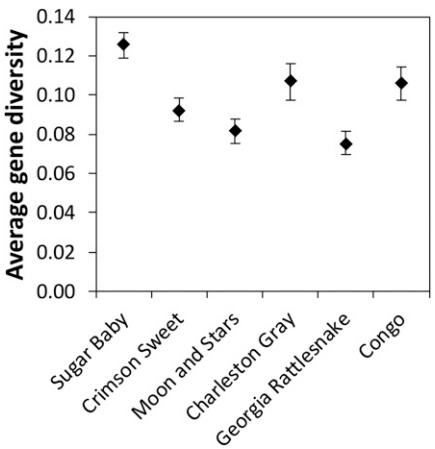

Fig. 2. The average gene diversity of six heirloom and open-pollinated watermelon cultivars using 32 simple sequence repeat loci. Gene diversity was calculated for each locus using the formula $D = 1 - \sum p_i^2$, where p_i is the frequency of i^{th} allele, then averaged over loci. Standard error bars were generated by bootstrapping the data 1000 times over loci.

Average gene diversity (Fig. 2), which accounts for both number and frequency of alleles, is a useful estimation for describing within-cultivar variation. 'Sugar Baby' had the greatest gene diversity; 'Charleston Gray' and 'Congo' were intermediate; and 'Crimson Sweet', 'Moon and Stars', and 'Georgia Rattlesnake' had the lowest. 'Sugar Baby' has significantly greater gene diversity than 'Moon and Stars (P = 0.0461). Low gene diversity estimates indicate that one allele per loci is predominant and alternative alleles occur at very low frequencies. One-third of loci were polymorphic across all six cultivars (data not shown); this variation may be attributed to residual variation from the progenitor gene pool, genetic drift, and de novo mutation.

Major patterns of variation in a pairwise individual × individual genetic distance matrix were calculated and plotted using PCoA (Supplemental Fig. 2). The arrangement of cultivar groups parallels the NJ tree (Fig. 1) and additionally reveals the variation among individuals in each cultivar as the spread of points in the group. 'Sugar Baby and 'Moon and Stars' are distantly related to the other cultivars, whereas overlap occurs among the other cultivar groups, particularly 'Charleston Gray' and 'Georgia Rattlesnake'. This is further evidence that 'Georgia Rattlesnake' was involved in the pedigree of 'Charleston Gray'. As expected, AMOVA revealed that more variation exists among cultivars (F_{ST} = 65%) than within cultivars and individuals (F_{IS} = 35%; Table 6).

Private alleles, which occur in one cultivar and no other, may be of interest to conservationists and breeders aiming to preserve and exploit diversity. As expected based on previous genetic distance estimates, 'Sugar Baby' has the most private alleles (PA = 14; Fig. 3). 'Crimson Sweet' and 'Moon and Stars' have eight and seven private alleles, respectively. 'Charleston Gray', 'Georgia Rattlesnake', and 'Congo' have relatively fewer private alleles, likely because of overlapping pedigrees. The abundant private alleles observed in 'Crimson Sweet' despite its close relation to other cultivars in the study may be attributed to the portion of its progenitor gene pool that excludes the other cultivars.

Genetic diversity among seed lots. A second level of analysis was conducted to characterize the extent of genetic variation among seed lots of each cultivar sourced from different commercial seed vendors. For this within-cultivar evaluation, each cultivar was analyzed as a separate data set.

There are differences in percentage of polymorphic loci and average number of alleles among seed lots in each cultivar (Supplemental Table 2), although gene diversity is fairly consistent (Fig. 4). One exception was a significant difference (P = 0.021) in gene diversity between 'Charleston Gray' seed lot #16 (D = 0.13) and seed lot #17 (D = 0.08). PCoA reveals no obvious clustering of individuals by seed lot (Supplemental Fig. 4). However, a significant difference among seed lots of 'Moon and Stars' is detected via AMOVA (Table 7). There was zero variation detected among seed lots of 'Georgia Rattlesnake'. For the remaining cultivars, 1% variation was detected among seed lots. Pairwise estimates of F_{ST} indicate that most seed lots are genetically similar (F_{ST} < 0.05) and in each cultivar group, there was at least one pair of seed lots that differed by less than 0.1% (Supplemental Table 3). Differentiation among seed lots of 'Moon and Stars' can mostly be attributed to the significant variation of seed lot #15 (F_{ST} > 0.05) from the others.

Although significant variation among seed lots was uncommon, almost all seed lots contained private alleles, defined as those alleles that occur in no other seed lot (Fig. 5). However, private alleles were observed in only one sampled individual in most cases (gray bars; Fig. 5), thus their practical use in breeding programs is limited.

Heirloom vs. modern OPs. The prevailing view of the grassroots "seed savers" movement is that heirloom cultivars are bastions of genetic diversity and are at risk of being lost and replaced by genetically less diverse modern varieties, although evidence points to the contrary (Heald and Chapman, 2012). Breeders and professionals involved with formal germplasm maintenance require genetic

Table 6. Analysis of molecular variance (AMOVA) among seed lots from various commercial seed vendors for six heirloom and modern open-pollinated watermelon cultivars based on 32 simple sequence repeat markers.

Source	df[z]	Variation (%)[y]	F-statistics[x]	P value[w]	Fst′[v]
Among cultivars	5	65%	0.649	0.001	0.772
Within cultivars	263	12%	0.337	0.001	
Within individuals	269	23%	0.767	0.001	

[z]Degrees of freedom.
[y]AMOVA was used to partition total variance into among group, within group, and within individual variance (Michalakis and Excoffier, 1996).
[x]F-statistics calculated via AMOVA (Nei, 1977) were used to estimate differentiation among groups (F_{ST}), within groups (F_{IS}), and within individuals (F_{IT}).
[w]The null hypothesis was tested using 999 random permutations of the data. F-statistics are significant at $P \leq 0.05$.
[v]F_{ST}' is F_{ST} standardized based on maximum F_{ST} possible in the data set (Meirmans, 2006).

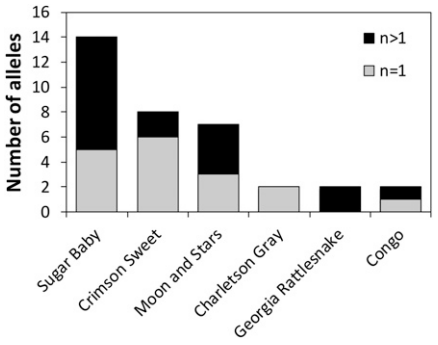

Fig. 3. Number of private alleles observed in in six heirloom and modern open-pollinated watermelon cultivars generated from the genotyping of 32 simple sequence repeat loci. Gray bars indicate private alleles that were found in only one individual; black bars indicate private alleles that were found in more than on individual.

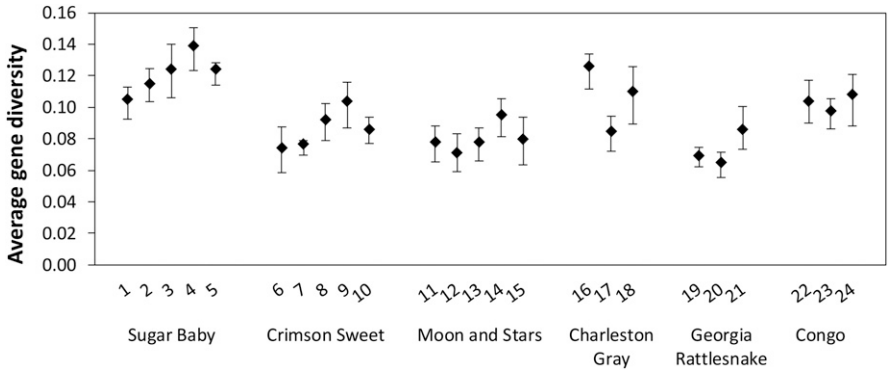

Fig. 4. The average gene diversity of seed lots from various commercial seed vendors for six heirloom and open-pollinated watermelon cultivars using 32 simple sequence repeat loci. Gene diversity was calculated for each loci using the formula D = 1 – pi2, where pi is the frequency of ith allele, then averaged over loci. Standard error bars were generated by bootstrapping the data 1000 times over loci.

evaluations to effectively prioritize conservation efforts. With these concerns in mind, an a priori comparison was used to determine if heirlooms 'Moon and Stars' and 'Georgia Rattlesnake' exhibit more genetic diversity than the modern cultivars featured in this study.

Intercultivar analysis revealed that Moon and Stars is genetically distinct from the other cultivars, whereas Georgia Rattlesnake is relatively similar to modern cultivar Charleston Gray (Supplemental Fig. 3). Thus, for these particular cultivars, genetic variation is most accurately attributed to pedigree rather than the "heirloom" designation per se. Furthermore, 'Moon and Stars' and 'Georgia Rattlesnake' exhibited the lowest average gene diversity (Fig. 2), which is an effective estimator for within-cultivar diversity. When exploring genetic diversity among seed lots, significant variation was observed in 'Moon and Stars', yet zero variation was detected in 'Georgia Rattlesnake' (Table 7). This result echoes the previous conclusion that the "heirloom" designation does not consistently correlate with genetic variation. Instead, variation among seed lots is likely a consequence of foundation seed maintenance practices. For example, 'Moon and Stars' is an heirloom of high consumer demand; it is possible that the sampled seed lots were multiplied from independent foundation seed lots. However, there is a lower demand for 'Georgia Rattlesnake' seed and thus the seed lots sampled in this study may be derived from a single foundation seed lot or even from a single multiplication plot. Unfortunately, requests from the seed companies for origin information that would confirm these inferences were largely unfulfilled. Nonetheless, our results suggest that both pedigree information and foundation seed maintenance practices should be considered when targeting cultivars and seed lots for conservation efforts rather than an heirloom designation.

The present investigation sought to characterize the genetic diversity of cultivars popular among U.S. organic, direct-market, and home growers, at both the cultivar and seed lot level, to better inform conservation and breeding efforts. Cultivars that contain distinct genetic resources, such as Sugar Baby and Moon and Stars, should be prioritized over cultivars that carry the "heirloom" designation per se. Breeders can use within-cultivar variation to maintain and improve elite cultivars for a changing climate (Tokatlidis, 2015). In this conservation breeding strategy, seed lots with above-average gene diversity, such as 'Charleston Gray' seed lot #16, should be prioritized over more genetically uniform seed lots, such as 'Charleston Gray' seed lot #17 (Fig. 4). Furthermore, when significant genetic variation occurs among seed lots, as was observed in 'Moon and Stars' in the present study, cultivar diversity is not fully captured by conserving one seed lot. Currently, the U.S. National Plant Germplasm System maintains a seed lot of 'Moon and Stars' deposited by Seed Savers Exchange in 2004. 'Moon and Stars' seed lot #15 was found to be significantly differentiated from the others (Supplemental Table 3), yet was phenotypically similar to other seed lots for major horticultural traits (Table 4; Supplemental Table 1). Therefore, the conservation of this differentiated seed lot, both via independent foundation seed maintenance and formal germplasm bank deposit, is warranted. In the case of heirloom 'Georgia Rattlesnake', no genetic variation was observed among seed lots. This suggests that one foundation seed lot is likely the source for commercial seed featured in this study and special attention should be given to conserve and properly maintain this genetic resource. The active maintenance and protection of genetic variation among seed lots may prove essential in the long-term conservation of these beloved heirloom cultivars.

Table 7. Analysis of molecular variance (AMOVA) among seed lots from various commercial seed vendors for six heirloom and modern open-pollinated watermelon cultivars based on 32 simple sequence repeat markers.

Cultivar	Source	df	Variation (%)[z]	F-statistic[y]	*P* value[x]	F_{ST}'[w]
Sugar Baby	Among seed lots	4	1%	0.006	0.264	0.007
	Within seed lots	52	13%	0.131	0.002	
	Within individuals	57	86%	0.136	0.002	
Crimson Sweet	Among seed lots	4	1%	0.009	0.131	0.010
	Within seed lots	52	52%	0.526	0.001	
	Within individuals	57	47%	0.531	0.001	
Moon and Stars	Among seed lots	4	4%	0.037	0.001	0.043
	Within seed lots	51	32%	0.332	0.001	
	Within individuals	56	64%	0.357	0.001	
Charleston Gray	Among seed lots	2	1%	0.006	0.314	0.007
	Within seed lots	28	16%	0.159	0.004	
	Within individuals	31	84%	0.164	0.003	
Georgia Rattlesnake	Among seed lots	2	0%	0.000	0.538	0.000
	Within seed lots	32	42%	0.417	0.001	
	Within individuals	35	58%	0.416	0.001	
Congo	Among seed lots	2	1%	0.008	0.239	0.010
	Within seed lots	30	42%	0.426	0.001	
	Within individuals	33	57%	0.430	0.001	

[z]AMOVA was used to partition total variance into among group, within group, and within individual variance (Michalakis and Excoffier, 1996).
[y]F-statistics calculated via AMOVA (Nei, 1977) were used to estimate differentiation among groups (F_{ST}), within groups (F_{IS}), and within individuals (F_{IT}).
[x]The null hypothesis was tested using 999 random permutations of the data. F-statistics are significant at $P \leq 0.05$.
[w]F_{ST}' is F_{ST} standardized based on maximum F_{ST} possible in the data set (Meirmans, 2006).

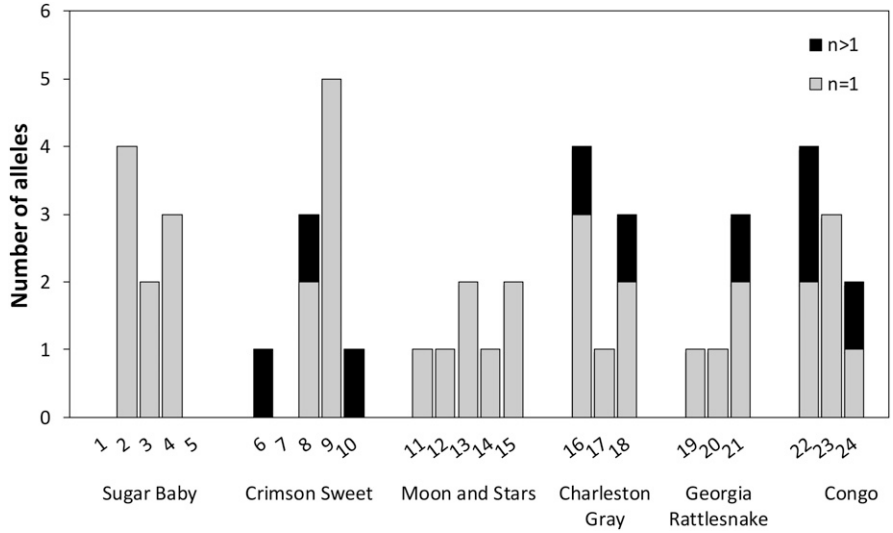

Fig. 5. Number of private alleles observed in seed lots from various commercial seed vendors generated from the genotyping of 32 simple sequence repeat loci in six heirloom and modern open-pollinated watermelon cultivars. Gray bars indicate private alleles that were found in only one individual; black bars indicate private alleles that were found in more than on individual.

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Supplemental Table 1. Mean phenotypic values of horticultural traits of seed lots from various commercial seed vendors for six heirloom and open-pollinated watermelon cultivars. Values within cultivars and columns with a different lowercase letter have means that are significantly different at $P \leq 0.05$.

		Fruit weight (kg)		Fruit length (cm)		Fruit width (cm)		Flesh firmness (kg·cm^{-2})	
Cultivar	Seed lot	2014	2015	2014	2015	2014	2015	2014	2015
Sugar Baby	1	2.9	5.1	20.6	21.9	19.1	20.7	1.6	1.5
	2	3.4	4.7	21.7	21.2	20.0	19.5	1.5	1.8
	3	2.9	4.9	21.3	21.7	19.4	20.1	1.5	1.5
	4	3.3	4.6	21.5	21.2	20.2	20.3	2.1	1.7
	5	3.4	4.7	19.7	21.3	18.6	20.3	1.7	1.8
Crimson Sweet	6	6.8	10.0	27.7	28.4	23.9	25.9	1.7	1.2
	7	6.9	9.3	27.8	28.1	24.3	25.0	1.5	1.2
	8	7.2	9.7	26.2	27.5	24.1	25.3	1.7	1.1
	9	9.0	9.0	26.9	28.5	24.3	24.5	1.7	1.3
	10	6.5	9.5	27.0	27.8	24.3	25.5	1.8	1.1
Moon and Stars	11	6.8	12.2	25.9	30.6	23.4	26.7	1.8	1.2
	12	7.0	12.2	26.7	30.2	24.0	27.3	1.9	1.2
	13	5.9	11.2	27.1	28.8	23.7	26.8	2.0	1.1
	14	6.1	12.0	25.9	28.8	23.6	26.5	2.0	1.3
	15	6.5	12.0	26.7	30.3	24.0	26.9	2.2	1.1
Charleston Gray	16	6.5	11.3	38.7	42.6	20.3	22.1	2.0	1.0
	17	8.2	8.1	41.3	37.5	19.8	19.7	2.0	1.1
	18	7.2	10.8	39.2	43.7	19.3	21.3	1.9	1.2
Georgia Rattlesnake	19	6.1	10.4	38.3	41.3	19.5	21.1	1.8	1.0
	20	7.4	10.5	39.6	41.1	19.1	21.4	2.1	1.2
	21	7.0	10.8	40.4	43.0	21.1	21.4	1.9	1.3
Congo	22	7.7	10.1	36.2	39.5	21.0	22.1	1.7	1.4
	23	7.0	11.0	37.0	39.3	21.9	22.6	2.0	1.4
	24	7.8	8.8	35.7	36.5	20.5	21.1	2.4	1.8

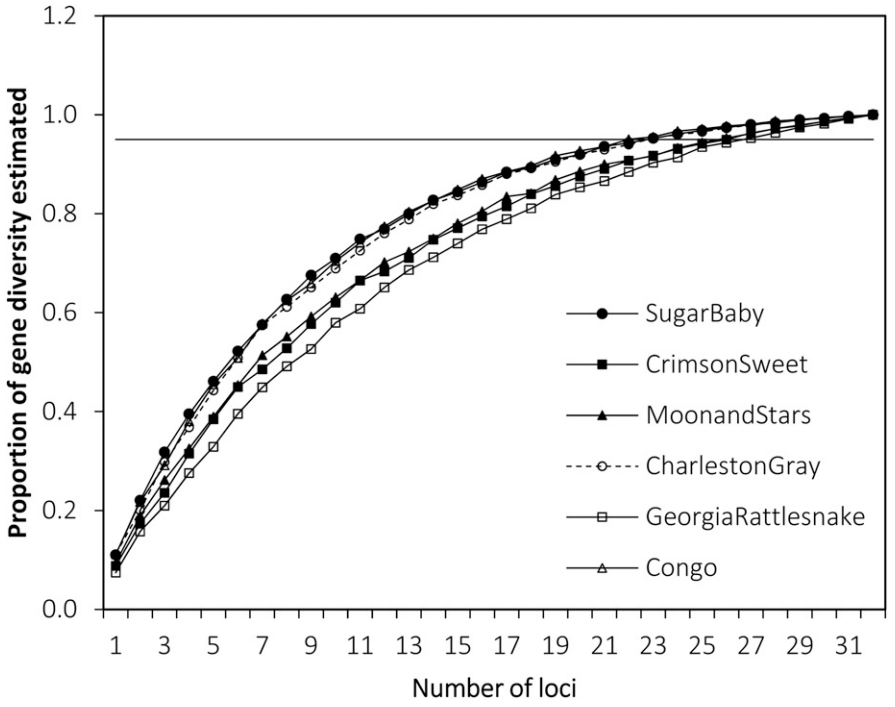
Supplemental Table 2. Genetic diversity parameters of seed lots from various commercial seed vendors for six heirloom and open-pollinated watermelon cultivars based on 32 simple sequence repeat loci.

Cultivar	Seed Lot	%P[z]	Na[y]
Sugar Baby	1	37.5	1.44
	2	46.9	1.53
	3	53.1	1.56
	4	59.4	1.59
	5	43.8	1.44
Crimson Sweet	6	31.3	1.31
	7	25.0	1.25
	8	34.4	1.38
	9	37.5	1.44
	10	28.1	1.28
Moon and Stars	11	34.4	1.41
	12	37.5	1.44
	13	31.3	1.38
	14	40.6	1.47
	15	34.4	1.38
Charleston Gray	16	40.6	1.47
	17	34.4	1.34
	18	46.9	1.50
Georgia Rattlesnake	19	28.1	1.28
	20	21.9	1.28
	21	37.5	1.44
Congo	22	43.8	1.50
	23	37.5	1.44
	24	40.6	1.41

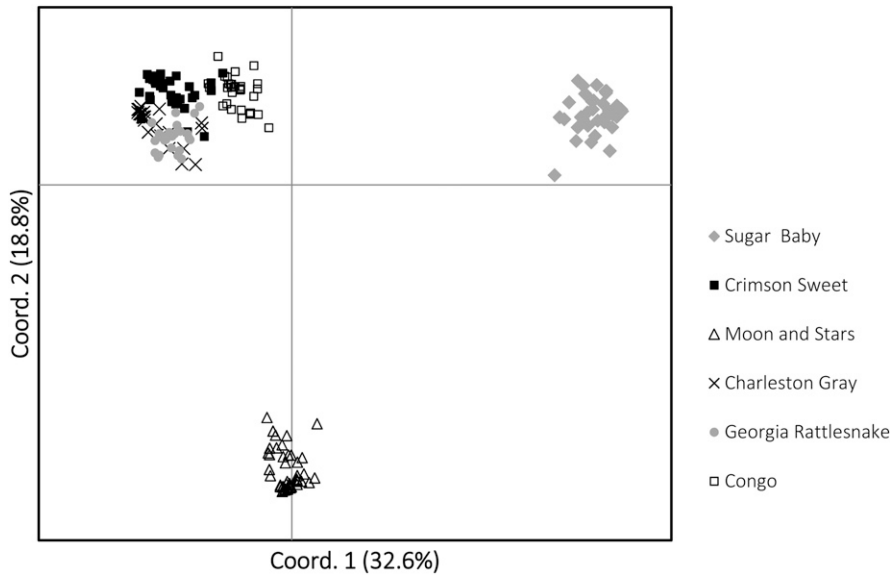
[z]Percent polymorphic loci (%P).
[y]Number of alleles, averaged over loci (Na).

Supplemental Table 3. Pairwise estimates of genetic differentiation (F_{ST}) between seed lots from various commercial seed vendors for six heirloom and modern open-pollinated watermelon cultivars. F_{ST} was calculating according to Nei (1977).

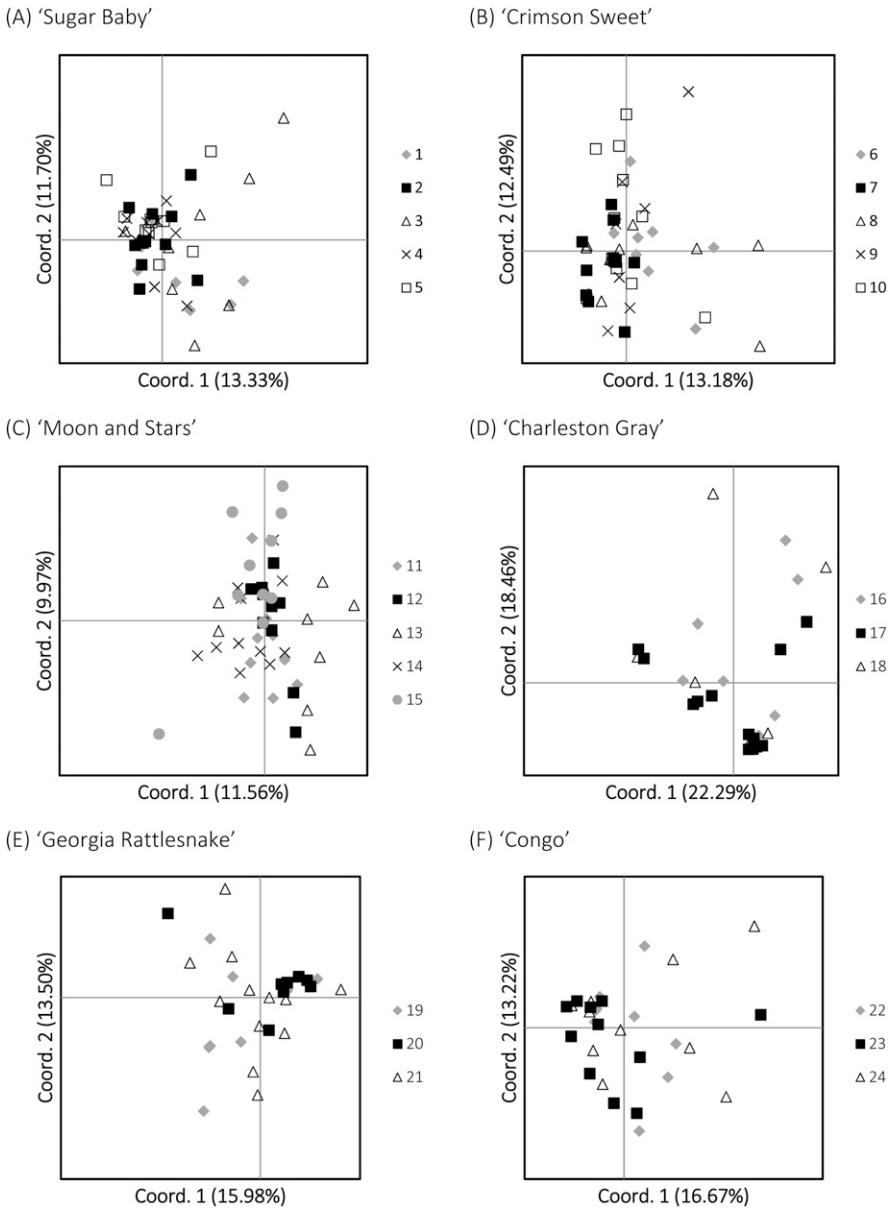
Cultivar	Seed Lot	1	2	3	4	5
Sugar Baby	1					
	2	0.002				
	3	0.000	0.005			
	4	0.005	0.002	0.014		
	5	0.032	0.009	0.020	0.000	
		6	7	8	9	10
Crimson Sweet	6					
	7	0.042				
	8	0.000	0.003			
	9	0.023	0.002	0.006		
	10	0.004	0.014	0.018	0.000	
		11	12	13	14	15
Moon and Stars	11					
	12	0.011				
	13	0.022	0.027			
	14	0.001	0.022	0.032		
	15	0.062	0.077	0.067	0.058	
		16	17	18		
Charleston Gray	16					
	17	0.039				
	18	0.001	0.000			
		19	20	21		
Georgia Rattlesnake	19					
	20	0.017				
	21	0.000	0.006			
		22	23	24		
Congo	22					
	23	0.027				
	24	0.000	0.000			



Supplemental Fig. 1. A power analysis of proportion of gene diversity estimated in six heirloom and open-pollinated watermelon cultivars vs. number of loci, as generated by 1000 random permutations of the data set of 32 simple sequence repeat loci.



Supplemental Fig. 2. A principal coordinate analysis using genetic distance among individuals of six heirloom and modern open-pollinated watermelon cultivars.



Supplemental Fig. 3. A principal coordinate analysis using genetic distance among individuals of seed lots from various commercial seed vendors for six heirloom and modern open-pollinated watermelon cultivars.