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ORIGINAL ARTICLE

Phytophthora colocasiae from Vietnam, China, Hawaii and Nepal: intra- and inter-genomic variations in ploidy and a long-lived, diploid Hawaiian lineage

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Abstract *Phytophthora colocasiae* is an important pathogen of taro and is widely distributed. Our goal was to develop whole genome sequence and single nucleotide polymorphism (SNP) markers to characterize historical and current populations from Hawaii (2010 and 2016, HA), historical isolates from Vietnam and China (2010, VN and CH) and current isolates from Nepal (2016, NEP). Seven isolates (VN = 2, CH = 1, HA = 1, NEP = 3) were sequenced (NCBI BioProject PRJNA378784) and compared using the reference genome of the closely related vegetable pathogen *P. capsici*. Genome-wide SNP analysis using 27,537 markers revealed genomes of diploid, triploid, tetraploid and higher ploidy. Ploidy varied within and between populations, with HA being primarily diploid, CH primarily triploid, VN containing diploid and triploid isolates, and NEP having predominantly triploid, tetraploid and higher ploidy. A total of 37 SNP markers were genotyped in 89 samples (grown in culture or directly from infected tissue) using targeted-sequencing. Analyses indicate a single clonal lineage dominated populations in HA from 2010 to 2016 and targeted-sequencing was useful to estimate ploidy. The implications for adaptation and evolution of *P. colocasiae* are discussed, as well as consequences for selection and breeding of resistant taro cultivars.

Keywords Ploidy · *Phytophthora colocasiae* · Taro · Oomycetes · SNP · Targeted sequencing

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Introduction

Taro (*Colocasia esculenta* (L.) Schott) is an important staple root crop with an estimated global production of 10.1 million metric tons in 2014 (FAO 2017). It is widely cultivated in Asia, Africa, South America, Caribbean and the Pacific islands (Kreike et al. 2004; Miyasaka et al. 2013). Taro belongs to the Araceae family and is mainly grown for the starchy corm, although the petiole and leaves, rich in fiber and vitamin C, are also eaten (Huang et al. 2000; Kreike et al. 2004; Miller 1927). Two botanical varieties, based on the shape of the corms, dasheen (var. *esculenta*) and eddoe (var. *antiquorum*), have been identified and are thought to be diploid and triploid, respectively (Irwin et al. 1998; Kreike et al. 2004).

Taro leaf blight (TLB) is caused by the oomycete plant pathogen *Phytophthora colocasiae* Raciborski (Raciborski 1900). Taro leaf blight lowers yield by damaging the photosynthetic area of the leaf and infects the petiole and corm (Brooks 2005). Initially, symptoms appear as small, dark brown flecks on the upper surface of leaves which rapidly expand to become circular, purplish brown to dark brown

Table 1 Summary of *Phytophthora colocasiae* isolates

Sample	Location	Genotype	DNA isolation
HI_LT8881	Hawaii	G1	Mycelium
HI_LT8873	Hawaii	G1	Mycelium
HI_LT8865	Hawaii	G1	Mycelium
HI_LT8857	Hawaii	G1	Mycelium
HI_LT8849	Hawaii	G1	Mycelium
HA_Bunlong_5	Hawaii	G1	Infected tissue
HA_255	Hawaii	G1	Infected tissue
HA_230	Hawaii	G1	Infected tissue
HA_176	Hawaii	G1	Infected tissue
HA_1025–96	Hawaii	G1	Infected tissue
HA_1025–87	Hawaii	G1	Infected tissue
HA_1025–82	Hawaii	G1	Infected tissue
HA_1025–81	Hawaii	G1	Infected tissue
HA_1025–80	Hawaii	G1	Infected tissue
HA_1025–79	Hawaii	G1	Infected tissue
HA_1025–64	Hawaii	G1	Infected tissue
HA_1025–60	Hawaii	G1	Infected tissue
HA_1025–58	Hawaii	G1	Infected tissue
HA_1025–56	Hawaii	G1	Infected tissue
HA_1025–510	Hawaii	G1	Infected tissue
HA_1025–502	Hawaii	G1	Infected tissue
HA_1025–473	Hawaii	G1	Infected tissue
HA_1025–399	Hawaii	G1	Infected tissue
HA_1025–382	Hawaii	G1	Infected tissue
HA_1025–379	Hawaii	G1	Infected tissue
HA_1025–37	Hawaii	G1	Infected tissue
HA_1025–35	Hawaii	G1	Infected tissue
HA_1025–332	Hawaii	G1	Infected tissue
HA_1025–312	Hawaii	G1	Infected tissue
HA_1025–302	Hawaii	G1	Infected tissue
HA_1025–297	Hawaii	G1	Infected tissue
HA_1025–291	Hawaii	G1	Infected tissue
HA_1025–287	Hawaii	G1	Infected tissue
HA_1025–283	Hawaii	G1	Infected tissue
HA_1025–274	Hawaii	G1	Infected tissue
HA_1025–269	Hawaii	G1	Infected tissue
HA_1025–255	Hawaii	G1	Infected tissue
HA_1025–250	Hawaii	G1	Infected tissue
HA_1025–242	Hawaii	G1	Infected tissue
HA_1025–239	Hawaii	G1	Infected tissue
HA_1025–229	Hawaii	G1	Infected tissue
HA_1025–220	Hawaii	G1	Infected tissue
HA_1025–2	Hawaii	G1	Infected tissue
HA_1025–19	Hawaii	G1	Infected tissue
HA_1025–188	Hawaii	G1	Infected tissue
HA_1025–187	Hawaii	G1	Infected tissue
HA_1025–186	Hawaii	G1	Infected tissue
HA_1025–181	Hawaii	G1	Infected tissue
HA_1025–180	Hawaii	G1	Infected tissue
HA_1025–168	Hawaii	G1	Infected tissue
HA_1025–13	Hawaii	G1	Infected tissue
HA_1025–125	Hawaii	G1	Infected tissue
HA_1025–124	Hawaii	G1	Infected tissue
HA_1025–122	Hawaii	G1	Infected tissue
HA_1025–118	Hawaii	G1	Infected tissue
HA_1025–100	Hawaii	G1	Infected tissue
HA_1024–215	Hawaii	G1	Infected tissue
HA_1005–66	Hawaii	G1	Infected tissue
HA_1005–35	Hawaii	G1	Infected tissue
HA_1003–3	Hawaii	G1	Infected tissue
VN_LT7573	Vietnam	G2	Mycelium
VN_LT7572	Vietnam	G2	Mycelium
VN_LT7571	Vietnam	G2	Mycelium
VN_LT7565	Vietnam	G2	Mycelium
VN_LT7564	Vietnam	G2	Mycelium
VN_LT7563	Vietnam	G2	Mycelium
CH_LT8549	China	G3	Mycelium
CH_LT8548	China	G3	Mycelium
CH_LT8541	China	G3	Mycelium
CH_LT8540	China	G3	Mycelium
Nepal-76	Nepal	G4	Infected tissue
Nepal-72	Nepal	G4	Infected tissue
Nepal-68	Nepal	G4	Infected tissue
Nepal-65	Nepal	G4	Infected tissue
HA_1025–51	Hawaii	G5	Infected tissue
HA_1025–240	Hawaii	G5	Infected tissue
VN_LT7581	Vietnam	G6	Mycelium
VN_LT7580	Vietnam	G6	Mycelium
CH_LT8525	China	G7	Mycelium
Nepal-10	Nepal	G8	Infected tissue
HA_1025–129	Hawaii	G9	Infected tissue
HA_1025–72	Hawaii	G10	Infected tissue
HA_1025–224	Hawaii	G11	Infected tissue
HA_1025–130	Hawaii	G12	Infected tissue
HA_2063–803	Hawaii	G13	Infected tissue
HA_1025–299	Hawaii	G14	Infected tissue
HA_1025–278	Hawaii	G15	Infected tissue
HA_1025–288	Hawaii	G16	Infected tissue
VN_LT7579	Vietnam	G17	Mycelium

lesions, often with concentric patterns. Lesions also have typical orange to red brown oozing, with prominent masses of white sporangia surrounding the edge (Brooks 2005; Nelson et al. 2011). *Phytophthora colocasiae* can produce spores sexually and asexually. The asexual sporangia can directly infect by germinating to produce a germ tube, or indirectly when swimming flagellated zoospores are released in water

Table 2 De novo contigs of *P. colocasiae* mapped to hypothetical linkage groups of *P. capsici;* the contigs are shown in the same order as they mapped to the linkage groups of *P. capsici*

Phytophthora capsici linkage groups	Size of LGs	*Phytophthora colocasiae* de novo contigs
LG01	6,673,488	369, 183, 712, 276, 746, 117, 636, 344, 521, 298, 518, 371, 707, 731, 272, 250, 437, 737, 464, 313, 2, 519, 397, 123, 708, 324, 678, 204, 676, 574, 734, 587, 477, 610, 699, 594, 396, 741, 744, 635, 666, 467, 148, 488, 192, 3
LG02	3,498,556	353, 201, 278, 51, 603, 11, 47, 474, 147, 616
LG03	3,505,123	215, 289, 709, 349, 404, 704, 639, 619, 642, 442, 392, 479, 227, 430, 649, 185, 317, 308, 783
LG04	2,121,855	498, 168, 169, 657, 62, 61, 615, 705, 173, 465
LG05	4,649,812	187, 328, 795, 791, 669, 152, 444, 46, 196, 202
LG06	2,252,950	391, 378, 158, 502
LG07	1,606,135	22, 634
LG08	3,373,591	454, 755, 773, 149, 393, 167, 684, 598, 459, 127, 341, 695, 501, 104, 798, 230
LG09	3,005,777	285, 362, 360, 198, 650, 779, 161, 786
LG10	6,004,071	766, 107, 162, 132, 421, 595, 282, 338, 419, 457, 1, 400, 64, 253, 75, 384, 140, 475, 539, 659, 259, 5, 38, 526, 243, 702, 468, 228, 662, 374, 677, 108, 380, 700, 320
LG11	4,095,992	790, 126, 570, 622, 67, 647, 153, 592, 247, 691, 646, 385, 606, 717
LG12	2,665,564	458, 71, 697, 747, 508, 453, 33, 443, 77, 522
LG13	2,711,105	65, 733, 480, 244, 199, 583, 590
LG14	2,028,471	239, 445, 626, 7
LG15	2,100,790	656, 164, 740, 359, 448
LG16	3,552,105	229, 780, 367, 93, 8, 210, 536, 194, 25, 690, 219, 777, 370, 491, 720, 383, 314, 120, 78, 195, 35, 297, 136, 572, 129
LG17	1,864,352	492, 234, 296, 277, 200, 711, 722, 382, 321
LG18	657,446	517, 409, 688, 617

(Brooks 2005). *Phytophthora colocasiae* is heterothallic, requiring the interaction of two mating types (A1 and A2) to produce thick-walled sexual oospores (Brooks 2005; Miyasaka et al. 2013; Nelson et al. 2011).

Taro leaf blight is globally distributed and has been found in Asia, Africa, South America, Oceania, Caribbean, and the Pacific territories (http://www.cabi.org/isc/datasheet/40955). For susceptible taro cultivars, yield reduction can be >50% and, in Hawaii, up to 95% leaf reduction has been reported (Nelson et al. 2011). The epidemic caused by TLB in American Samoa in the mid-1990s resulted in dramatic reduction of taro production and decimation of the local susceptible commercial cultivar (Brooks 2005; Miyasaka et al. 2013). Similarly, in 2009, TLB caused a drastic reduction in yield and loss of a susceptible commercial taro cultivar in the Dominican Republic (Miyasaka et al. 2013). Most of the world's taro production occurs in Africa, and production there decreased from approximately 9.6 million to 6.9 million tonnes between 2008 and 2010 (FAO 2017). This decline in production corresponds with the first reports of TLB occurring in Nigeria and Ghana in 2009 (Bandyopathy et al. 2011; Omane et al. 2012). Nigeria is the world's leading taro producer, and production during those years fell from 5.4 million tonnes to 2.9 million tonnes. Integrated approaches are used to control *P. colocasiae* including crop rotation, field sanitation, selection of disease-free vegetative-propagules, pesticides, and TLB-resistant cultivars (Miyasaka et al. 2013; Nelson et al. 2011; Uchida et al. 2002).

The diversity of *P. colocasiae* has been characterized previously using mating type, proteins, and genetic markers. These include a report of the A1 mating type ($n = 144$) recovered from taro on the islands of Hawaii, Maui and Kauai and the A2 mating type ($n = 799$) from Taiwan and, in both cases, the pathogen is presumed to be introduced (Ann et al. 1986; Ko 1979). On Hainan Island, China three mating types (A1, A2, and A0-neuter) were reported, and the authors suggest an Asian origin of *P. colocasiae* (Zhang et al. 1994). A study of 54 isolates from the Pacific regions, India, and South-east Asia revealed A2 and A0 mating types (Tyson and Fullerton 2007). A recent survey shows that Hawaii and Vietnam have A1 and A2 mating types with A2 dominating more than 95% of isolates. In contrast, A1:A2:A0 mating types from Hainan Island, China, are in the ratio of 69:27:4% (Shrestha et al. 2014). Isozyme and RAPD (Random Amplified Polymorphic DNA) revealed high genetic variation for isolates among and within five countries (Lebot et al. 2003).

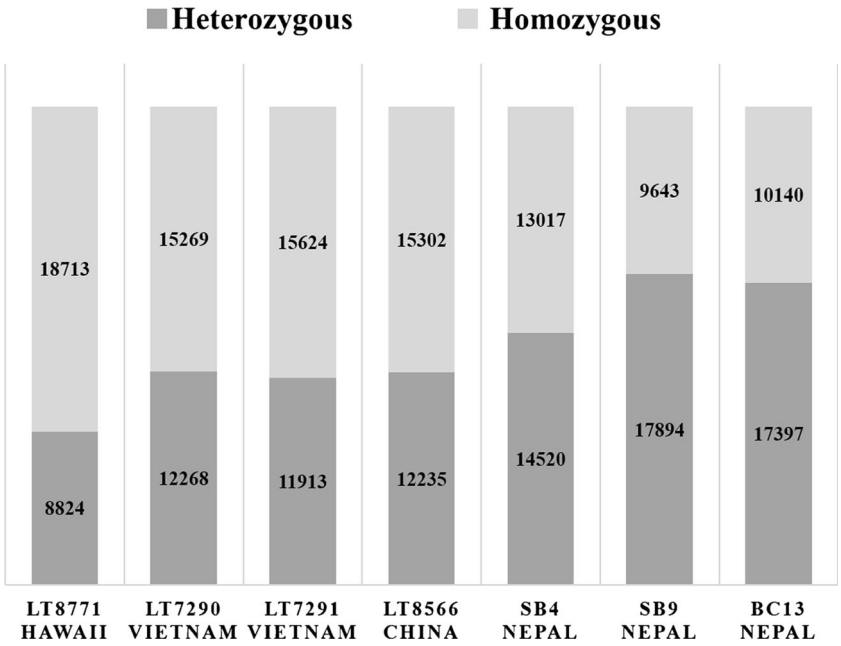


Fig. 1 Number of homozygous and heterozygous out of 27,537 SNP loci from whole-genome analysis of seven isolates against the de novo reference

Mishra et al. (2010) reported unique profiles for 14 isolates analyzed with isozyme and RAPD. Similarly, fine-scale sampling of *P. colocasiae* from multiple lesions on individual taro leaves in India revealed high levels of genotypic diversity using RAPD and AFLP (Amplified Fragment Length Polymorphism) markers and a surprisingly high level of sequence variation in ITS1 (internal transcribed spacer 1) region, although the authors indicate that the entire ITS1, 5.8S and ITS2 sequence confirms that all isolates are *P. colocasiae* (Nath et al. 2013a; Nath et al. 2013b). Characterization of populations on the Hawaiian islands, Vietnam and Hainan Island, China, using High Resolution DNA Melting (HR-

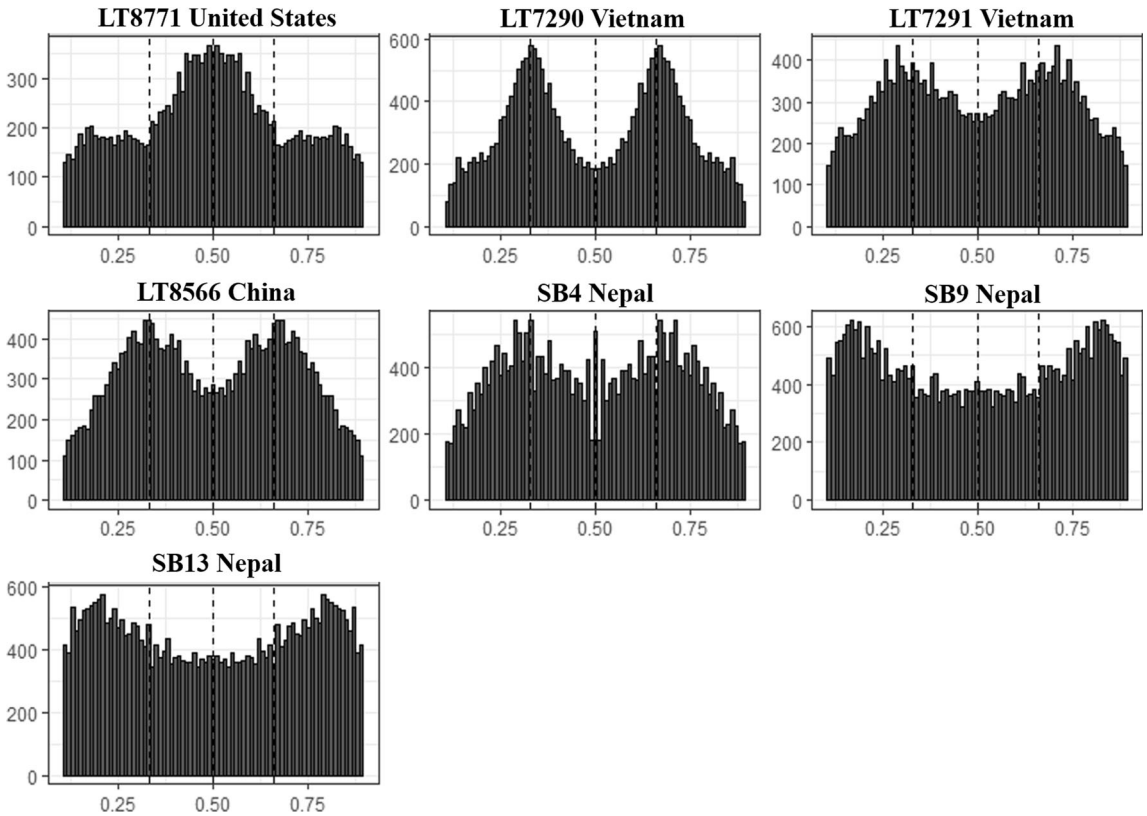


Fig. 2 Histograms showing intragenomic heterozygous allele frequencies for the heterozygous loci in Fig. 1

DM) analysis suggested clonal lineages predominate and some clonal lineages are shared among countries (Shrestha et al. 2014).

Genetic sequencing is providing unprecedented characterization of genetic variation and is useful to measure allele dosage (ploidy) which is difficult to assess using technologies that measure variation indirectly, such as HR-DMA and AFLP (e.g., via fluorescent signals or presence/absence of fragments in a gel matrix). Our previous work using HR-DMA to characterize populations of *P. colocasiae* revealed instances of Loss of Heterozygosity (LOH) where biological replications (mycelium grown in separate wells but derived from the same isolates) produced heterozygous and homozygous genotypes, similar to what was reported for the closely related vegetable pathogen, *P. capsici* (Shrestha et al. 2014). How the phenomenon of LOH works is unknown, although recent reports of triploid clonal lineages of *P. infestans* switching to the diploid state under conditions of stress suggest changes in ploidy may underlie observed LOH and may be part of the evolutionary strategy that makes this group of organisms difficult to study and successful as plant pathogens (Li et al. 2016).

Our initial goal was to assess genome diversity for *P. colocasiae* recovered from four countries to develop robust SNP markers useful for population analyses. As this work progressed, it became obvious that allele dosage (ploidy) was not homogenous across the *P. colocasiae* genome and our efforts shifted to focus on the assessment of intra- and inter-genomic variation in ploidy and the implications for genomic instability.

Materials and methods

Sample collection and DNA extraction

For isolates grown in culture, approximately 10-mm sections of taro leaves with typical TLB lesions were excised and placed onto V8-RAP plates (rifampicin 25 ppm, ampicillin 100 ppm, PCNB 25 ppm, 160 mL unfiltered V8 juice, 20 g agar, 3 g calcium carbonate and 840 mL water). A hyphal tip was transferred to V8-RAP agar and, after 3–5 days, a tuft of mycelium was transferred to V8-RAP liquid broth for 5–7 days for mycelium production. Mycelium was lyophilized and genomic DNA extracted using a standard phenol–chloroform extraction method. In addition, genomic DNA was extracted directly from infected tissue. For infected tissue samples, four 7-mm discs were punched from the edge of a distinct lesion using a disposable plastic punch (a section of drinking straw) and then placed into a single well of a 2-ml 96-well plate containing 3–5 3-mm glass beads, freeze-dried, and genomic DNA was extracted as previously described (Lamour and Finley 2006).

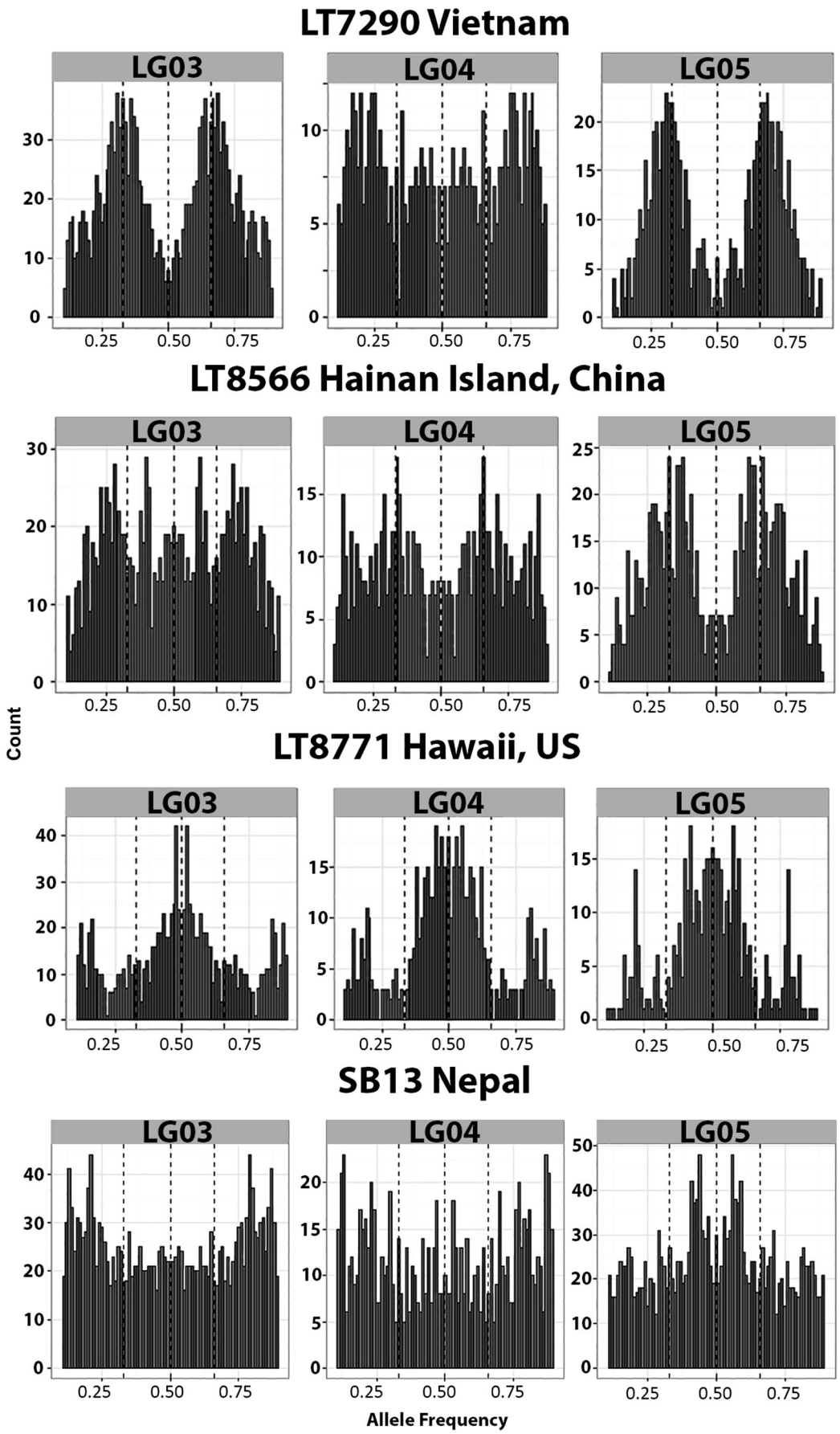
Whole genome sequencing and development of de novo reference sequences

Genomic DNA was sheared to 200 bp using a Covaris M220 focused-ultrasonicator (Covaris, Woburn, MA, USA). PCR-free Illumina libraries were built using the KAPA Hyper Prep Kit and the resulting libraries quantified using the KAPA Library Quantification Kit (Kapa Biosystems, Wilmington, MA, USA). Libraries were sequenced at the Oklahoma Medical Research Facility on an Illumina HiSeq3000 device running a 2 × 150 paired-end configuration. The resulting sequences were trimmed based on quality using CLC Genomics Workbench 9.5.2 (CLC-GW) (CLC Bio, Aarhus, Denmark) and processed further to develop *P. colocasiae*-specific reference contigs and to identify putative SNP sites for population analyses.

A set of nuclear genomic reference sequences for *P. colocasiae* was developed by de novo assembly of *P. colocasiae* using CLC-GW at default settings, except only contigs >10Kbp were retained. The resulting contigs were mapped to 18 reference sequences derived from the *P. capsici* reference genome that contain only those contigs/scaffolds able to be assigned to linkage groups. The *P. colocasiae* contigs able to be mapped were annotated with open reading frames (ORFs) greater than 300 amino acids using CLC-GW and referred to as the PcoloREF.

Single nucleotide polymorphism (SNP) discovery and target selection

To identify candidate SNP sites, whole genome sequences were mapped to the PcoloREF requiring 90% of a read to have 90% identity, and BAM files exported for further processing using the Genome Analysis Toolkit (GATK) (McKenna et al. 2010). Genotypes were assigned using the diploid HaplotypeCaller followed by hard filtering as recommended by the developers, and custom Perl script was used to extract data for sites with a minimum of ×20 coverage (https://github.com/sandeshsth). Genotypes were assigned as homozygous for alleles at <10% and >90% and heterozygous for alleles between 10 and 90%. A subset of putative SNPs that fall into ORFs and are predicted to be silent were selected for targeted-sequencing and subsequent genotyping. The SNP site in the PcoloREF was changed to an 'N' and the flanking sequences extracted as a multi-FASTA file using custom Perl scripts (https://github.com/sandeshsth). Generic primers were designed using BatchPrimer3 v.1.0 (You et al. 2008). Amplification of the target regions and PCR-free library construction (as described above) was conducted by Floodlight Genomics (Knoxville, TN, USA) as part of a no-cost Educational and Research Outreach Program. Each sample had two technical replications and the resulting sample-specific sequence data was made available by FTP transfer.


LT7290 Vietnam
LG03
LG04
LG05
LT8566 Hainan Island, China
LG03
LG04
LG05
Count
LT8771 Hawaii, US
LG03
LG04
LG05
SB13 Nepal
LG03
LG04
LG05
Allele Frequency

◀ **Fig. 3** Representative intragenomic variation in ploidy for *P. colocasiae* within potentially linked markers. Data are shown for linkage groups 3, 4 and 5 (based on the linkage groups of *P. capsici*)

Sample-specific sequences were mapped to the extracted target sequences and processed as above using CLC-GW and GATK.

Genetic analysis

A phylogenetic tree was constructed using all putative silent SNPs across the PcoloREF using the maximum parsimony method with 1000 bootstraps in MEGA7 (Kumar et al. 2016). The initial tree was generated by random addition of sequences (100 replicates) using the Subtree–Pruning–Regrafting (SPR) algorithm with search level 1 (Nei and Kumar 2000). One *P. capsici* isolate was included as an outgroup. For the isolates and infected plant samples with targeted-sequencing data, samples with identical multi-locus genotypes were identified and a representative genotype retained for further analysis. Allele frequency histograms were constructed using the heterozygous loci from whole genome sequences or targeted sequencing using ggplot2 (Wickham 2009). In addition, for isolates with Whole Genome Sequence (WGS), separate histograms were constructed based on the 18 linkage groups reported for the *P. capsici* genome.

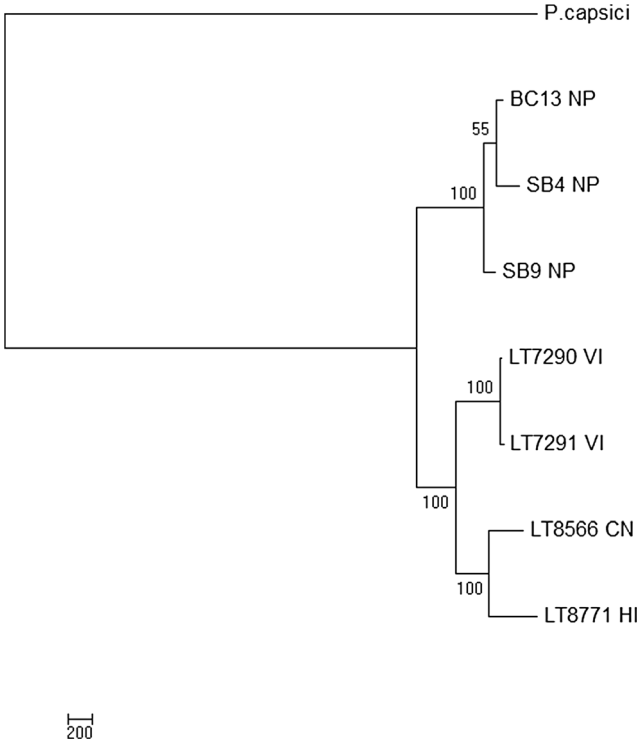


Fig. 4 Maximum parsimony tree constructed with 8230 silent SNPs

Results

Isolates

In total, 89 individual isolates of *P. colocasiae* from Nepal (5), Hawaii (70), China (5) and Vietnam (9) were included; for DNA extraction, mycelium was used for 19 isolates and infected tissue was used for rest of the 70 isolates (Table 1).

Genome sequencing and ploidy

The following seven isolates were sequenced: China (LT8566), Hawaii (LT8771), Nepal (SB4, SB9, and BC13), and Vietnam (LT7290 and LT7291). A total of 42.6 (LT8771), 25.7 (LT8566), 61.7 (LT7290), 39 (LT7291), 14.5 (SB4), 17.92 (SB9) and 24.7 (BC13) million 151-bp paired-end reads were produced and the sequences were deposited in the National Center for Biotechnology Information (NCBI) as BioProject PRJNA378784. The Hawaiian isolate, LT8771, was de novo assembled to produce the PcoloREF. The de novo assembly produced 800 contigs >10Kbp, with an average size of 17,003 bp and N50 of 17,006. A total of 238 contigs (3.8Mbp) mapped to the 18 linkage groups of *P. capsici* (Table 2). A total of 27,537 putative SNPs were identified in the seven isolates (average of 1 SNP every 138 bp). The proportion of heterozygous loci ranged from 17,894 in the Nepalese isolate, SB9, to 8824 in the Hawaiian isolate, LT8771 (Fig. 1).

Histograms based on the full complement of intragenomic heterozygous allele frequencies revealed distinct (and indistinct) distributions with some isolates appearing to be primarily diploid (Hawaii), triploid (China, Vietnam and Nepal) or some higher level of ploidy (Nepal) (Fig. 2). If ploidy is consistent across the entire genome, distinct modal distributions centering on 50% for diploids, 33 and 66% for triploids, 25, 50 and 75% for tetraploids, etc. are expected. Histograms constructed based on grouping the PcoloREF contigs according to the 18 linkage groups of *P. capsici* indicate that ploidy is not consistent within a genome (Fig. 3; Supplementary Fig. 1).

Interestingly, the triploid and higher ploidy isolates had between 14 and 32% more heterozygous loci compared to the diploid isolate from Hawaii. Phylogenetic analysis with 8230 silent (synonymous mutation, does not change the amino acid) SNPs grouped isolates into three clades with isolates from China and Hawaii grouped separately from Vietnam, and these groups being distinct from Nepal where the higher ploidy isolates were more similar than the triploid Nepalese isolate (Fig. 4).

Targeted sequencing and genotype analyses

In total, 37 SNP markers were assayed in 89 isolates of *P. colocasiae* from four different countries. The information

about the contig, position, and primers of SNP markers is listed in Tables 3 and 4. Multi-locus SNP analysis and clone correction produced seventeen unique genotypes. The genotypes were assigned from G1-G17 (Table 5). Countries did not share genotypes, and Hawaii was dominated by a single clone, G1, with 60 isolates. Although there were many fewer markers, the histograms produced using the heterozygous allele frequencies for the 37 markers provided a reasonable estimate of the predominate ploidy for an isolate (Fig. 5).

Discussion

Our goal was to develop genomic and genetic resources for *P. colocasiae* which would be useful for characterizing populations. Once whole-genome and targeted sequencing data were produced; ploidy became the focus of our analyses. It varies within and between countries and varies at different sites within individual isolates. The finding of higher ploidy, especially the triploid state, is not new for the genus *Phytophthora*, and the late

Table 3 Summary data for putative silent single nucleotide polymorphism markers assayed in populations

SNP ID	REF	ALT	LT7290 Vietnam	LT7291 Vietnam	LT8566 China	LT8771 Hawaii, US	*P. capsici* linkage group
Pcolo_684_13931	A	G	AG	AG	AA	AG	LG08
Pcolo_676_3466	G	A	GG	GG	GA	GA	LG01
Pcolo_370_11738	A	G	GG	GG	AG	AG	LG16
Pcolo_702_7135	G	A	AA	AA	AA	GA	LG10
Pcolo_740_5719	C	T	CT	CT	CC	CC	LG15
Pcolo_308_10962	G	A	GG	GG	GG	GA	LG03
Pcolo_380_11300	T	C	TC	TC	CC	TC	LG10
Pcolo_362_8339	G	A	GA	GA	GA	GG	LG09
Pcolo_444_6155	A	G	AA	AA	AG	AG	LG05
Pcolo_71_3603	A	G	AG	AG	GG	AG	LG12
Pcolo_791_4647	T	C	TC	TC	CC	TC	LG05
Pcolo_321_8314	C	T	CT	CT	CT	CC	LG17
Pcolo_634_3078	C	T	CT	CT	CT	CC	LG07
Pcolo_464_10426	A	G	AG	AG	AG	AA	LG01
Pcolo_790_7563	A	G	GG	GG	GG	AG	LG11
Pcolo_198_6620	C	T	CT	CT	CT	CC	LG09
Pcolo_239_12371	C	T	CC	CC	CC	CT	LG14
Pcolo_474_7932	T	G	GG	GG	TG	TG	LG02
Pcolo_786_6148	G	A	GA	GA	AA	GA	LG09
Pcolo_454_19141	C	T	CT	CT	TT	CT	LG08
Pcolo_169_7557	A	G	AG	AG	AG	AA	LG04
Pcolo_35_6639	T	C	TC	TC	TC	TT	LG16
Pcolo_369_4359	C	T	TT	TT	TT	CT	LG01
Pcolo_210_1616	C	T	CC	CC	CT	CT	LG16
Pcolo_353_4747	G	A	GA	GA	GA	GG	LG02
Pcolo_229_13053	T	C	TC	TC	CC	TC	LG16
Pcolo_717_9721	C	T	CC	CC	CC	CT	LG11
Pcolo_183_15469	A	G	AG	AG	GG	AA	LG01
Pcolo_282_6878	T	C	TT	TT	TC	TC	LG10
Pcolo_3_12979	G	T	GT	GT	GT	GG	LG01
Pcolo_296_7034	G	A	GA	GA	AA	GA	LG17
Pcolo_247_9704	G	A	GA	GA	GA	GG	LG11
Pcolo_67_16737	T	C	TC	TC	CC	TC	LG11
Pcolo_230_4547	C	T	CT	CT	CC	CC	LG08
Pcolo_202_13537	C	G	CG	CG	CC	CG	LG05
Pcolo_7_16284	G	A	GG	GG	GG	GA	LG14
Pcolo_656_11915	C	T	CT	CT	CC	CT	LG15

Table 4 Primers used to amplify 60-70 bp regions containing SNP markers

SNP ID	Forward 5′–3’	Reverse 5′–3’
Pcolo_684_13931	GGCGATGAACTCGTCGAT	CGTGAGAACCAGGAGCA
Pcolo_676_3466	TTGGGAACTTGGTGGA	AGCTCGGTCTCGGTGTT
Pcolo_370_11738	GAAGAAGCGGCGAATTGA	CAGCACTCCTCGACCTCA
Pcolo_702_7135	GAAAAGTTTGCACTTCG	TGCCATCGAGAGACTCCA
Pcolo_740_5719	GGATGAACAGGCGGAACA	AACAAAGCGGACACA
Pcolo_308_10962	AGCTATCACCCACGTGCAG	CGAGGTTCACACCACGACT
Pcolo_380_11300	TGCCGATGTCGGTCACT	AATGAGATCGGACCAACGA
Pcolo_362_8339	TCGACGTGAGTTCGTCGAT	CGGACACAGCGTCACGTA
Pcolo_444_6155	TGCATCGACCTTTCAGGA	CACCGTTTGTCCCCACTC
Pcolo_71_3603	GCGTATTCAGGAACCTGTAGTT	TTCGTGAAGCAGGTCCTTTT
Pcolo_791_4647	AGATCTGTCATCAACCTCCAA	CAAAGACTAACCGCTTGTATTCAC
Pcolo_321_8314	GGCCACGGCAAACACT	CTTTGGGGTGGACACAA
Pcolo_634_3078	GTGGCATGTGGTGGTGAG	TCACCGTAGCTGTAAACGTCTC
Pcolo_464_10426	TGGGTGACAAGACGGTCA	ATCGGCGTCATGCTTTGT
Pcolo_790_7563	ATGGGAATTGGCCTCCTAA	ACCCTGGAAATCGCTGAG
Pcolo_198_6620	TGTTGAACGTGCCGTTGT	CAAGTGACGTGTATTCGTTCAT
Pcolo_239_12371	AACGATCATCGAACAAGG	TTCTGCAGGGCGAAGAG
Pcolo_474_7932	GCCAAGAGCCAGCAACTC	AGTACATTGCTGCTCGATT
Pcolo_786_6148	TGCGAAAGTGTTTACCATCA	CTCGACACGGACGGAGAC
Pcolo_454_19141	TGCGGAACGTATCCAACC	CTTTTCAAAGCGCCACT
Pcolo_169_7557	GGCGATCTCGCTGTTGTT	TTCCAGCGCCAGGACT
Pcolo_35_6639	GCCTTGGAAGCGTTCTTG	TTCGAATCCGGAAGCTG
Pcolo_369_4359	AAAAACCTCCGGTCCAG	ATCGATCGTCGCACCAAC
Pcolo_210_1616	GAGCACTGGTGGTACGC	ACTGATCATGGCGACGAAA
Pcolo_353_4747	AGAGCTCCCAGGACCACA	CAAGTTGACGTTCGGTTCC
Pcolo_229_13053	GGCCTCCAGCTCCAGTTC	CAGTGAGCATGAGCAGCAA
Pcolo_717_9721	TTAGCTTCGTCCACATT	TGAGGCTCGTGTGGATCA
Pcolo_183_15469	GCGCGACGAGAGGAGAAT	CAAAAACCTCGTTTTCC
Pcolo_282_6878	GTGGTGGAAGGCACT	CATCCAGGCAAGCACGTA
Pcolo_3_12979	AATCCACGTCAACACG	TGGATTCTGGCGGACCTA
Pcolo_296_7034	GGCGGTACTCTTTCACG	TGTGAAGAACTACACGAAAAAGG
Pcolo_247_9704	TGCTGCTGACTGCGGATA	CGAGTATGGCGGTTCCTG
Pcolo_67_16737	GCTTTTGTGCCGGTAAT	TCTCTTCCGGTTCGTAAAGG
Pcolo_230_4547	TTCGCCTTCCTTCTCCAA	AACAGGCAAAGGAACTCCAG
Pcolo_202_13537	TCCAGGCTTTCCATGTTGA	CAAGATGACCCGGGAGAA
Pcolo_7_16284	GATTTCTGCGTCTGCAA	TGCTGAAATTCGCAAAGGA
Pcolo_656_11915	ACCGGTTGAGTCGCTGTG	AAGGACGTGAAGAAGTGGGTTA

blight pathogen, *P. infestans*, is often comprised of a few widely dispersed and long-lived triploid clonal lineages. What is new is the detection of intragenomic variations in ploidy for *P. colocasiae*. We are documenting this same phenomenon, also using WGS and targeted sequencing, of inter- and intra-genomic variability in ploidy for isolates of *P. capsici* from natural field populations in Taiwan and for single-zoospore isolates from *P. capsici* recovered from China (unreported data).

It is becoming apparent that the ability to tolerate changes in ploidy may play an important role in the success of asexual lineages for *Phytophthora*. In *P. infestans*, most successful clonal lineages are triploid, can persist many years, are dispersed over broad geographical regions, and can vary in ploidy (e.g., triploid reduced to diploid) under stress (e.g., fungicides or starvation) (Li et al. 2016). A recent report on *P. capsici* isolates recovered from a multi-year, closed field experiment in New York tracked an inbreeding population using >20 K SNP markers produced using the Genotype by Sequencing (GBS) method and also found isolates with increased ploidy, although the exact level of ploidy was difficult to estimate (Carlson et al. 2017). The authors found a mating-type region (MTR) that retained significantly higher levels of

Table 5 Unique genotypes identified by multi-locus analysis of 37 SNP markers. Markers are arranged in order as presented in Table 3

Genotype ID	Genotype	Country	Number of isolates
G1	AGGAGAAGCCGATCGGAGGACTCCCCAAGACTCTGTAGCTAATTTCCTGGTCCTAATCGGGAGGCTCCCGGACT	Hawaii	60
G2	AGGGGGAACTGGTCGAAAGACTCTTCAGGGCTCCGGAGCTAGTCTTCCGATCCCGATTTGGAGACTCTCGGGCT	Vietnam	6
G3	AAGAGAAACCGGCCGAAGGGCCCTTCAGGGCTCCGTAATTAGTCTTCTGACCCCGGTCTGAAGACCCCCCGGCC	China	4
G4	AAGGGGAACCGGTTGGAGGGCCCTTTAAGGTTCCGGAGCCAATCTCCCGACCCCGATTGGGGGGCCCC CCGGCT	Nepal	4
G5	AGGAGAAGCCGATCGAAGGACTCCCCAAGACTCTGTAGCTAATCTCCTGGTCCTGATCGGGAGGCTCCCGGACT	Hawaii	2
G6	AGGAGAAGCCGATCGGAGGACTCCCCAAGACTCTGTAGCTAATTTCCTGGTCCCAATCGGGAGGCTCCCGGACT	Vietnam	2
G7	AAGAGAAACCGGCCGAAGGGCCCTTCAGGGCTCCGTAATTAGTCTTCTGACCCCGGTTTGAAGACCCCCCGGCC	China	1
G8	AAGAGGAACCGGTCGAAGGGCCCCTCAAGGCTCCGGAGCTAACCCCCCGACCCCGGTTGGGAGGCCCC CCGGTT	Nepal	1
G9	AGGAGAAACCGATCGAAGGACTCTTCAGGACTCTGTAGCTAGTCTCCCGATCCCGATCTGGAGGCTCCCCGGCT	Hawaii	1
G10	AGGAGAAACCGGTCGAAGGACTCTTCAAGACTCTGTAGCTAATCTCCCGATCCCGATCTGGAGGCTCCCGGACT	Hawaii	1
G11	AGGAGAAGCCGATCGAAGGACTCCCCAAGACTCTGTAGCTAATCTCCTGGTCCTGATCGGGAGGCTCCCGGACC	Hawaii	1
G12	AGGAGAAGCCGATCGGAGGACTCCCCAAGACTCTGTAGCTAATCTCCTGGTCCTAATCGGGAGGCTCCCGGACT	Hawaii	1
G13	AGGAGAAGCCGATCGGAGGACTCCTCAAGACTCTGTAGCTAATCTCCTGGTCCTGATCGGGAGGCTCCCGGACT	Hawaii	1
G14	AGGAGAAGCCGATCGGAGGACTCTTCAAGACTCTGTAGCTAATCTCCTGGTCCTGATCGGGAGGCTCCCGGACT	Hawaii	1
G15	AGGAGAAGCCGATCGGAGGACTCTTCAAGACTCTGTAGCTAATCTCCTGGTCCTGATCTGGAGGCTCCCGGACT	Hawaii	1
G16	AGGAGGAGCCGGTCGAAGGGCCCCCCAAGACTCTGGAGTTAATCTTCCGGCCCCGATTGGGAGGCCCC CCGGCC	Hawaii	1
G17	AGGGGGAGCCGGTCGGAGGGCCCTTCAAGACTCCGGAACCAATTTCCCGGTCCCGATTTGGGGGCTCCCCGGTT	Vietnam	1

heterozygosity, despite inbreeding, and was associated with the A2 mating type. Since *P. colocasiae* and *P. capsici* are obligately outcrossing organisms, variable ploidy occurring across a similar MTR may allow *P. colocasiae* to generate both mating types from single isolates. Taiwan and Vietnam were dominated by a single mating type, whereas Hainan Island had an equal distribution of both mating types (Ann et al. 1986; Shrestha et al. 2014; Zhang et al. 1994).

Previous work indicated that *P. colocasiae* exists primarily as asexual clones in Hawaii, Vietnam, and China and, in general, country-specific clones dominate, although some clonal lineages were shared between countries (Shrestha et al. 2014). Previous

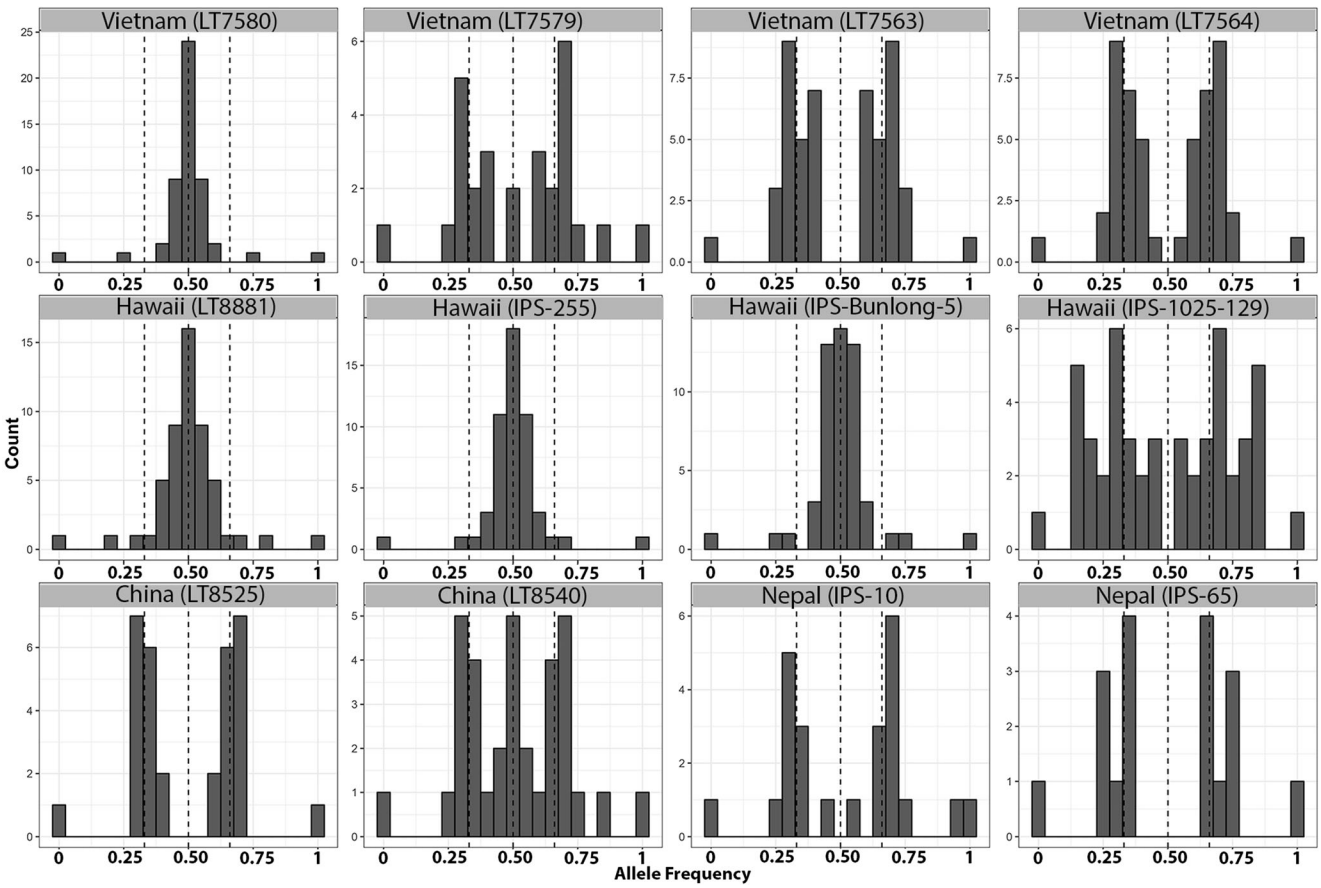


Fig. 5 Representative histograms showing heterozygous alternate allele frequencies for the 37 SNP loci genotyped by targeted-sequencing for isolates grown in culture (*LT*) or from infected plant samples (*IPS*) from Vietnam, Hawaii, China and Nepal

studies also showed that the asexual reproduction is favorable, and that a single mating type, either A1 or A2, dominated the fields (Ann et al. 1986; Ko 1979). The ability to accommodate many intragenomic levels of ploidy provides an additional level of plasticity that may be highly useful for adaptation and surmounting obstacles (e.g., novel resistant hosts or chemicals). During favorable conditions, it could remain at higher ploidy for asexual reproduction and rapid distribution and during adverse conditions, switch to the diploid state to generate both mating types to allow sexual recombination and the production of thick-walled oospores for extended survival outside the plant host (Berman and Hadany 2012; Li et al. 2016). A potentially similar situation occurs under stress in *Candida albicans* in which concerted chromosome loss in tetraploid zygotes produces diploid strains (Alby and Bennett 2009). Higher ploidy, such as that found in the Nepalese isolates, may explain why some isolates are not able to produce oospores when paired with either A1 or A2 mating types (Shrestha et al. 2014; Tyson and Fullerton 2007; Zhang et al. 1994).

An organism with higher ploidy has a higher preserved variation compared to their diploid counterpart, and this was the case with *P. colocasiae* where the tetra- or higher ploidy isolates carried more heterozygosity compared to the triploid or diploid isolates. Similarly, triploid *P. infestans* isolates had higher levels of heterozygosity compared to diploid, including areas of the genome with RXLR and Crinkler (CRN) effector genes (Li et al. 2016). Increased ploidy in genes undergoing positive selection pressures may impact adaptation and vigor, as an increase in beneficial mutations and faster adaptation are reported for tetraploid yeast compared to haploid and diploid counterparts (Selmecki et al. 2015).

Ko suggested that *P. colocasiae* originated in Asia where there are diverse wild and cultivated taro cultivars (Ko 1979). Interestingly, the isolates from Nepal had higher amounts of intragenomic genetic variation compared to isolates from Hawaii, Vietnam and China. The higher ploidy for isolates from Nepal may be necessary, as taro is widely distributed with different shapes (single-corm, multi-corm, and multi-cormel), and is found in high mountain, hill and plain (terai) regions (Pandey et al. 1998; Rijal et al. 2003). The locally cultivated taro cultivars are diverse and wild-types are common and popular as pig fodder (Pandey et al. 1998). Additional sampling in Nepal will be helpful to better understand the situation.

This study provides important new insight into the kinds of genomic variation possible with *P. colocasiae*. The potential for intragenomic variation in ploidy, beyond triploid to tetraploid and likely even higher levels, adds a powerful new dimension to the capabilities of *P. colocasiae*, even if it is existing primarily in the clonal state. Taro leaf blight is an important disease and ongoing work to identify and introgress resistance genes may be impacted by the plasticity of the *P. colocasiae* genome, especially in cases where single or a few isolates are used to screen promising germplasm. Clearly, much additional work is needed to fully understand the implications for intragenomic variations in ploidy on disease development, evolution, and the development of effective control measures.

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References

Alby K, Bennett RJ (2009) Stress-induced phenotypic switching in *Candida albicans*. Mol Biol Cell 20:3178–3191

Ann P, Kao C, Ko W (1986) Mating-type distribution of *Phytophthora colocasiae* in Taiwan. Mycopathologia 93:193–194

Bandyopathy R, Sharma K, Onyeka TJ, Aregbesola A, Kumar PL (2011) First report of taro (*Colocasia esculenta*) leaf blight caused by *Phytophthora colocasiae* in Nigeria. Plant Dis 95:618. doi:10.1094/PDIS–12–10–0890

Berman J, Hadany L (2012) Does stress induce (para) sex? Implications for *Candida albicans* evolution. Trends Genet 28:197–203

Brooks F (2005) Taro leaf blight. The Plant Health Instructor. doi:10.1094/PHII2005053101

Carlson MO, Gazave E, Gore MA, Smart CD (2017) Temporal genetic dynamics of an experimental, biparental field population of *Phytophthora capsici*. Front Genet 8. doi:10.3389/fgene.2017.00026

FAO (2017) FAOSTAT. Available at http://www.fao.org/faostat/en/%23home

Huang A, Titchenal C, Meilleur B (2000) Nutrient composition of taro corms and breadfruit. J Food Compos Anal 13:859–864

Irwin SV, Kaufusi P, Banks K, de la Peña R, Cho JJ (1998) Molecular characterization of taro (*Colocasia esculenta*) using RAPD markers. Euphytica 99:183. doi:10.1023/a:1018309417762

Ko W (1979) Mating–type distribution of *Phytophthora colocasiae* on the island of Hawaii. Mycologia 71:434–437

Kreike CM, Van Eck HJ, Lebot V (2004) Genetic diversity of taro, *Colocasia esculenta* (L.) Schott, in Southeast Asia and the Pacific. Theor App Genet 109:761–768. doi:10.1007/s00122–004–1691–z

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874

Lamour K, Finley L (2006) A strategy for recovering high quality genomic DNA from a large number of *Phytophthora* isolates. Mycologia 98:514–517

Lebot V, Herail C, Gunua T, Pardales J, Prana M, Thongjiem M, Viet N (2003) Isozyme and RAPD variation among *Phytophthora colocasiae* isolates from south-east Asia and the Pacific. Plant Pathol 52:303–313

Li Y, Shen H, Zhou Q, Qian K, van der Lee T, Huang S (2016) Changing ploidy as a strategy; the Irish potato famine pathogen shifts ploidy in relation to its sexuality. Mol Plant Microbe In 30:45–52

McKenna A et al (2010) The genome analysis toolkit: a MapReduce framework for analyzing next–generation DNA sequencing data. Genome Res 20:1297–1303

Miller CD (1927) Food values of poi, taro, and limu. Bull Bishop Mus 37:1–25

Mishra AK, Sharma K, Misra RS (2010) Isozyme and PCR-based genotyping of epidemic *Phytophthora colocasiae* associated with taro leaf blight. Arc Phytopathol PFL 43:1367–1380

Miyasaka SC, Lamour K, Shintaku M, Shrestha S, Uchida J (2013) Taro leaf blight caused by *Phytophthora colocasiae*. In: Lamour K (ed) Phytophthora: a global perspective, 1st edn. CABI, Wallingford, pp 104–112

Nath VS, Senthil M, Hegde VM, Jeeva ML, Misra RS, Veena SS, Raj M (2013a) Genetic diversity of *Phytophthora colocasiae* isolates in India based on AFLP analysis. Biotech 3:297–305

Nath VS, Senthil M, Hegde VM, Jeeva ML, Misra RS, Veena SS, Raj M (2013b) Molecular evidence supports hypervariability in *Phytophthora colocasiae* associated with leaf blight of taro. Eur J Plant Pathol 136:483–494

Nei M, Kumar S (2000) Molecular evolution and phylogenetics. Oxford University Press, Oxford

Nelson S, Brooks F, Teves G (2011) Taro leaf blight in Hawaii. Plant disease bulletin no PD–71, University of Hawaii, Manoa

Omane E, Oduro KA, Cornelius EW, Opoku IY, Akrofi AY, Sharma K, Kumar PL, Bandyopadhyay R (2012) First report of leaf blight of taro (*Colocasiae esculenta*) caused by *Phytophthora colocasiae* in Ghana. Plant Dis 96:292. doi:10.1094/PDIS–09–11–0789

Pandey YR, Rijal DK, Sthapit B (1998) Diversity of taro and on-farm conservation through use in Nepal. Ethnobotany and genetic diversity of Asian taro with focus on China. In: Zhu D, Zhou M, Pablo E (eds) Proceedings of the symposium on ethnobotanical and genetic diversity study of taro in China: approaches for the conservation and use of taro genetic resources, 10–12 November, 1998. Laiyang Agricultural College, Laiyang, pp 18–25

Raciborski M (1900) Parasitic algae and fungi, java. Batavia Bull N Y State Mus 19:189

Rijal DK, Sthapit BR, Rana RB, Jarvis DI (2003) Adaptation and uses of taro diversity in agroecosystems of Nepal in: Sthapit B, Upadhyay M, Baniya B, Subedi a, Joshi B (eds) on–farm management of agricultural biodiversity in Nepal. Proceedings of National Workshop, 24–26 April 2001, Lumle, Nepal, pp 29–36

Selmecki AM et al (2015) Polyploidy can drive rapid adaptation in yeast. Nature 519:349–352

Shrestha S, Hu J, Fryxell RT, Mudge J, Lamour K (2014) SNP markers identify widely distributed clonal lineages of *Phytophthora colocasiae* in Vietnam, Hawaii and Hainan Island, China. Mycologia 106:676–685

Tyson J, Fullerton R (2007) Mating types of *Phytophthora colocasiae* from the Pacific region, India and south–east Asia. Aust Plant Dis Notes 2:111–112

Uchida JY, Silva JA, Kadooka CY (2002) Improvements in taro culture and reduction in disease levels. Plant disease bulletin no PD–22. University of Hawaii, Manoa

Wickham H (2009) ggplot2: elegant graphics for data analysis. Springer, New York

You FM, Huo N, Gu YQ, Luo M, Ma Y, Hane D, Lazo GR, Dvorak J, Anderson OD (2008) BatchPrimer3: a high throughput web application for PCR and sequencing primer design. BMC Bioinformatics 9:253. doi:10.1186/1471–2105–9–253

Zhang K, Zheng F, Li Y, Ann P, Ko W (1994) Isolates of *Phytophthora colocasiae* from Hainan Island in China: evidence suggesting an Asian origin of this species. Mycologia: 108–112