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Assessment of the genetic diversity of *Ulex europaeus* in Maui, California, Hawaii and New Zealand by a method of microsatellite markers

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Abstract: One of the serious invasive, allohexaploid plant species, *Ulex europaeus* is originally from Western Europe and spreading to the world by some unknown pathways. Plants often show phenotypic plasticity according to the environments, but making it clear that the differences are derived from environmental or genetic effects is very important for further study. Thus, the aim of this study was to assess the genetic distances of *Ulex europaeus* from four different regions such as Maui, California, Hawaii and New Zealand. Microsatellite method which has been used frequently to test the genetic distances of the hexaploid plant species recently was used for the assessment because normal single nucleotide polymorphism (SNP) often shows genotypic ambiguity on hexaploids. We tested the leaf samples of 37 mother trees from four regions (Maui: 11, California: 4, Hawaii: 7, New Zealand: 15) at five microsatellite loci. After polymerase chain reaction analyses (PCR), dinucleotide-repeat motifs (DRMs) were counted and compared to test the genetic distances of the samples. As a result, dendrogram and analysis of molecular variance (AMOVA) showed that *Ulex europaeus* sampled in four different regions were genetically very close. If they show any morphological differences, they are inferred to be derived from environmental effects.

Keywords:1; allohexaploid 2; genentic distances 3; invasive species 4; microsatellite 5; phenotypic plasticity

Ulex europaeus

100 noxious species by IUCN

because …

Strong **adaptation and propagation** ability

by producing **huge amount of seeds** in the pods

↓

Hinder the growth of native species

↓

Change the ecosystem

↓

Ecologically and economically troublesome

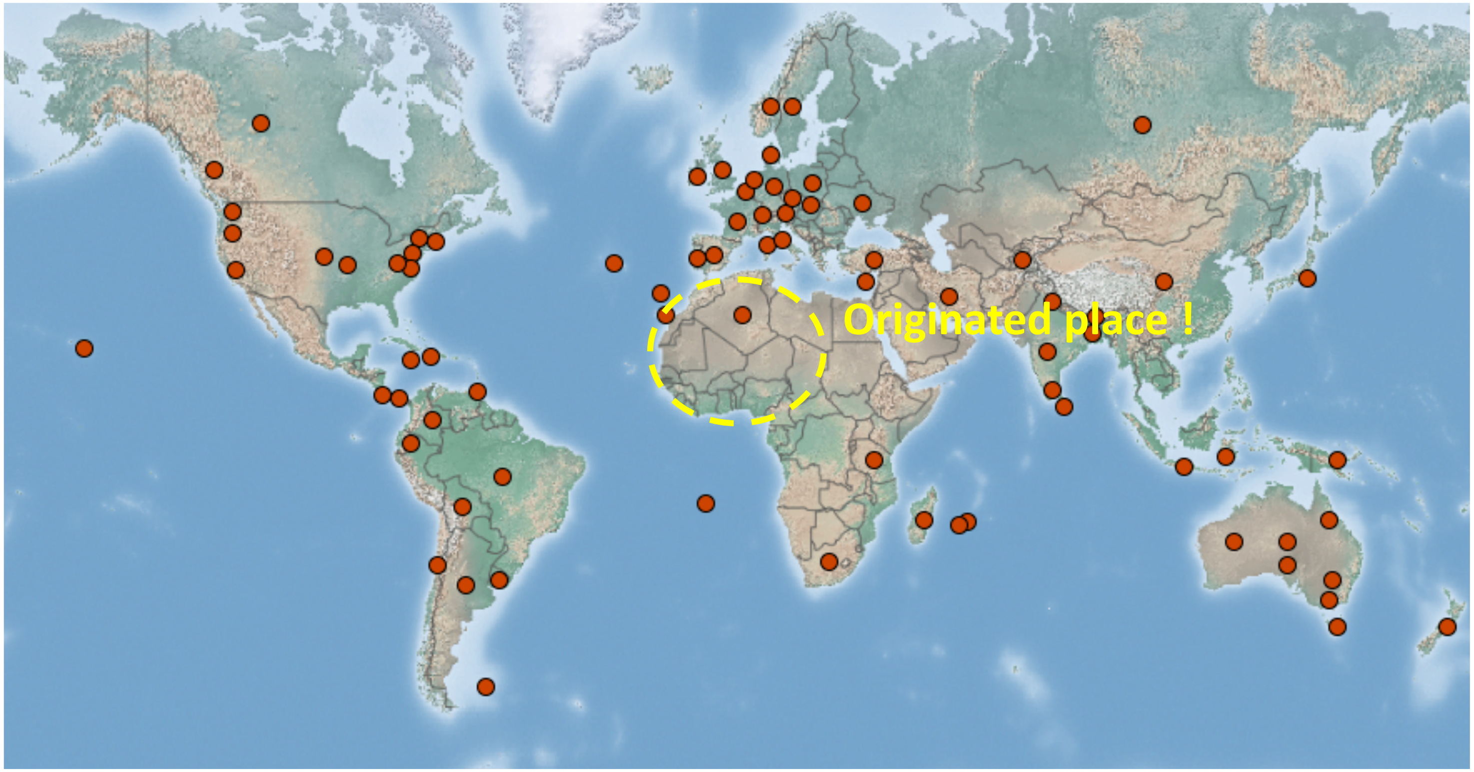
but…

Very difficult to control !!

8/1/2019 Hawaii Island
Mauna Kea

11/11/2017 New Zealand
Akaroa

Distribution map of *Ulex europaeus* in the world



Invade and adapt even in the tropics…

Invasive Species Compedium

(https://www.cabi.org/isc/datasheet/55561, browsed on 10/1/2019)

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Research questions of this research is ………

Morphological differences may
depend on the genetic differences??

★ Need to assess the **genetic distances** of
U. europaeus samples
taken for this study

Are they far or close？

Materials and Methods

Sampling sites…three regions, 36 mother trees, time…July 2016 to November 2017

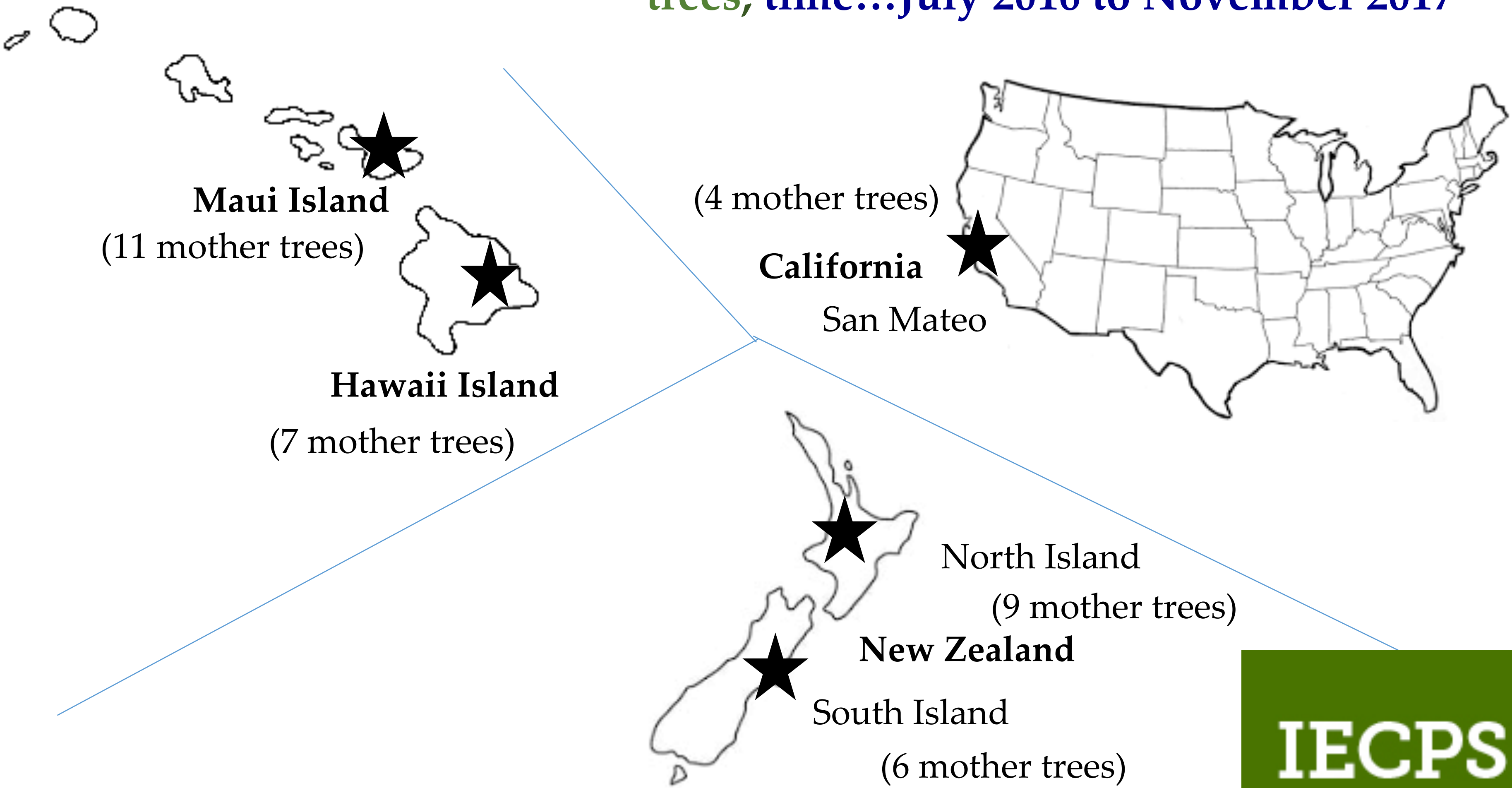

Figure 1. Sampling sites

Table 1. Location and coordinates of *Ulex europaeus* sampled for PCR experiments.

Location	Mother tree	Latitude	Longitude	Altitude (m)	Habitat
Maui	#22	N20.76	W156.27	1758	Ranch
	#23	N20.76	W156.27	1823	Ranch
	#26	N20.77	W156.26	1767	Ranch
	#27	N20.77	W156.26	1763	Ranch
	#28	N20.77	W156.26	1763	Ranch
	#29	N20.78	W156.25	1773	Ranch
	#30	N20.78	W156.25	1768	Ranch
	#33	N20.79	W156.25	1656	Ranch
	#35	N20.8	W156.28	1060	Forest
	#36	N20.8	W156.28	1067	Forest
	#37	N20.8	W156.28	1046	Forest
California	#40,41,42,43	N37.15	W122.34	33, 33, 34, 31	Fallow land
Hawaii	#49	N19.72	W155.44	2004	Ranch
	#50	N19.72	W155.43	2058	Ranch
	#51	N19.93	W155.41	2015	Ranch
	#52	N19.73	W155.39	1952	Ranch
	#52-2	N19.73	W155.39	1952	Ranch
	#53	N19.74	W155.37	1929	Ranch
	#55	N19.77	W155.36	1980	Ranch
New Zealand	N2-1, 2, 3	S36.88	E174.84	0	Fallow land
	N5-1, 2, 3	S37.86	E175.82	97	Roadside of a ranch
	N8-1, 2, 3	S38.99	E175.76	538	Fallow land with grass
	S1-1, 2, 3	S43.8	E173	623	Ranch
	S4-1, 2, 3	S43.5	E172.52	38	Roadside of a ranch

Tissue samples of the leaves → Scratched on FTA® Plant Card (Whatman® Co., Ltd.) for **PCR** (Polymerase chain reaction)

Table 2. Five microsatellite loci used in the experiments

Locus	Primers	[Primers](μM)
A110	F: 5′-CTATGGTGAATTTGTGATACAC-3′	0.35
	R: 5′-ACCTTGTTGCATCTTTACC-3′	
A125	F: 5′-GCATATACATACCCGAGGTAAG-3′	0.26
	R: 5′-AACCTGATGAAATGCACTATTC-3′	
B4	F: 5′-GGGCTCTGGCTCTGATAC-3′	0.2
	R: 5′-TTGGATTAACCAACTTTCCTC-3′	
B104	F: 5′-GAACCTTATTCACTGGAATCTG-3′	0.3
	R: 5′-CCCTTTTCTTTCCTTTCTTAAC-3′	
B123	F: 5′-AATTTGCCTGACATTGTTACTC-3′	0.22
	R: 5′-AGACCGTGTTCATTATGGTTAG-3′	

(Hornoy et al., 2013)

PCR

Enzyme……………KOD FX Neo of TOYOBO Co., Ltd.

Amount tested……25μL (12.5μL of buffer, 2.5μL of dNTP,
9μL of deionized and sterilized water,
0.4μL each of Primers (F and R), and 0.2μL of enzyme)

In iCycler (Bio Rad Laboratories, California, U.S.A) setting

Initial denaturation step at 94℃ for 2 minutes,

35 cycles with 10 seconds denaturation at 98℃,

30 seconds hybridization of primers at 60℃

1 minute elongation at 68℃,

Final elongation step at 68℃ for 7 minutes,

Check the condition of the bands at **electrophoresis** with 2% of agarose gel

↓

Next generation sequencing of the PCR products
by Takara Bio Inc. (Kusatsu, Shiga, Japan).

FASTA data were checked with software ApE (Utah University, free software)

Dinucleotide-repeat motifs (DRMs) of GT/TG, CT/TC, AG/GA, AG/GA, AG/GA （most common motifs found in five loci respectively) were counted by each locus using a software SEAVIEW（Guoy et al., 2010)

Because….

DRMs are the enhancers that express gene (Yanez-Cuna et al., 2014)

Analyses

Genetic diversity among and within populations was estimated by the analysis of molecular variance with the software GenAlEx (Peakall and Smouse, 2006; 2012).

Cluster dendrogram was calculated and indicated by software R ver. 3.3.2.

Results

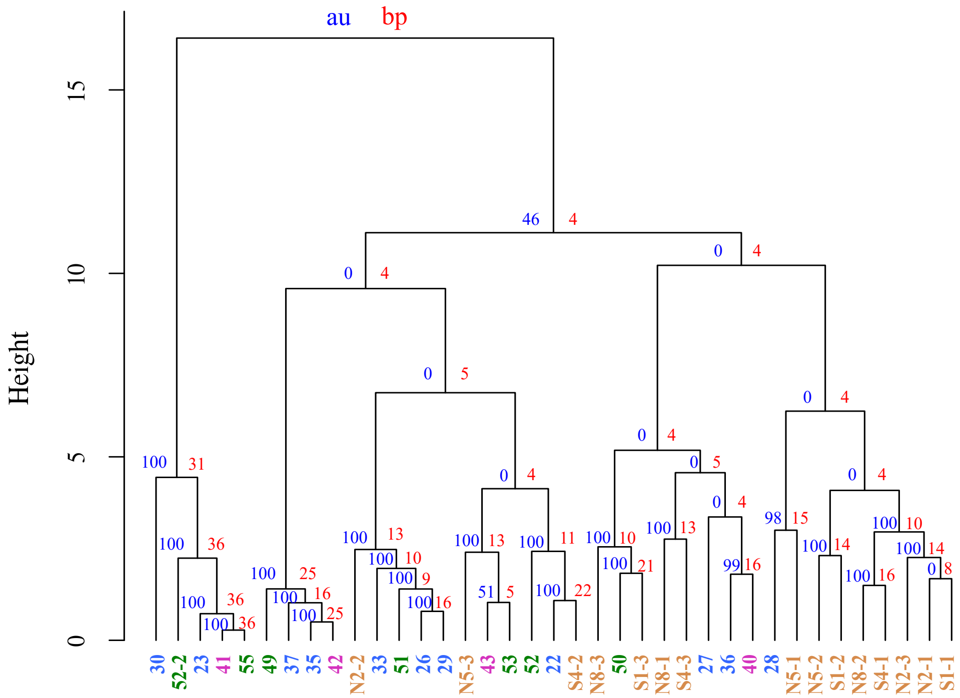

Figure 2. Dendrogram of *Ulex europaeus* sampled in Maui, California, Hawaii and New Zealand after 1000-boot strap. NN: NZL North Island, NS: NZL South Island.

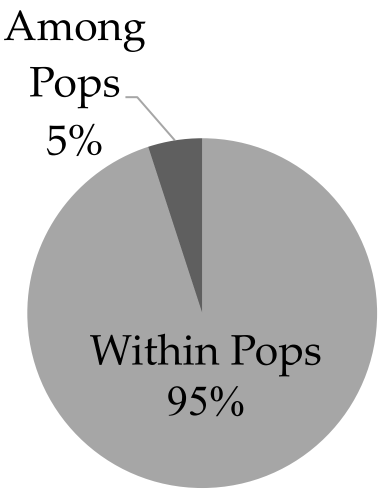

Figure 3. Percentages of Molecular variance among and within populations by AMOVA.

Not making specific group by population

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Discussions

U. europaeus samples taken from 3 regions

From the results of **cluster analysis**

★ All the mother trees were **genetically mostly the same**

From the results of **AMOVA**

★ **Molecular variance** was mainly occurred **within populations**: 95% of the total variations.

Inferred that they were genetically very close

Conclusion

The genetic distances of the samples taken from Maui, California, Hawaii, New Zealand were…

very close

Morphological differences, if any, are inferred to be derived from environmental effects such as climatic and geological effects

Benchmark of the further study

Supplementary Materials

Table 4. Results of the molecular variance of *U. europaeus* sampled in 3 regions for this study. Probability, P (rand >= data), for PhiPT is based on standard permutation across the full data set.

Source	**df**	**SS**	**MS**	**Est. Var.**	**%**
Among Pops	4	2960.2	740.05	27.79	5%
Within Pops	32	17276.99	539.91	539.91	95%
Total	36	20237.19		567.69	100%
Stat	Value	P (rand >= data)			
PhiPT	0.05	0.11			

Pescadero, California, USA (16 Jul/16)

Akaroa, NZL, South Island (11 Nov/17)

Along Saddle Road, Mauna Kea, Hawaii (17 Feb/20)

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Springfield, Canterbury, NZL South Island (12 Nov/17)

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Manaaki Whenua
Landcare Research

agresearch
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Haleakala Ranch
• Est. Maui 1888 •

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