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Geographic Structure of a True Pantropical Plant with Sea-drifted Seeds, *Ipomoea pes-caprae* (*Convolvulaceae*), Revealed by Nuclear Markers

Matin MIRYEGANEH[a], Koji TAKAYAMA[b], Yoichi TATEISHI[c] and Tadashi KAJITA[a,*]

[a]Department of Biology, Graduate School of Science, Chiba University, 1-33, Yayoi, Inage, Chiba, 263-8522 JAPAN;
[b]The University Museum, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo, 113-0033 JAPAN;
[c]Faculty of Education, University of the Ryukyus, 1, Senbaru, Nishihara, Okinawa, 903-0129 JAPAN
*Corresponding author: tkaji@faculty.chiba-u.jp

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"Pantropical plants with sea-drifted seeds" exhibit extremely wide distribution in the tropics and subtropics of the world, and the wide distribution range were attributed to long distance seed dispersal by ocean currents. We performed phylogeographic analyses on *Ipomoea pes-caprae* (*Convolvulaceae*) that is one of the 'true-pantropical' species, using two low copy nuclear genes, *HSP-90* and *Waxy*. Using 272 samples collected from 34 populations throughout the global distribution range, seven and five haplotypes were recognized in *HSP-90* and *Waxy* respectively, and the geographic distributions of haplotypes showed clear geographic structure. Several haplotypes were distributed in AEP (Atlantic – East Pacific) or IWP (Indo – West Pacific) regions almost specifically, while some haplotypes were very widespread across AEP or IWP regions. The presence of the same haplotypes over populations isolated by the ocean suggests that long distance dispersal by ocean currents is responsible for gene flow within the wide distribution range. Both the East Pacific and African Continent acted as geographic barriers that have prevented seed dispersal. Presence of more haplotypes in Africa or Indian Oceanic regions may suggest that the origin of the species was in these regions and that the species expanded its distribution toward the New World.

Key words: Long distance dispersal, phylogeography, sea-dispersal.

"Pantropical plants with sea-drifted seeds" are a group of plants consisting of littoral species which are distributed in an extremely wide distribution range across tropical and subtropical areas worldwide (Takayama et al. 2006, 2008, Vatanparast et al. 2011). Members of this plant group are characterized by their sea-drifted seeds that can be easily conveyed over long distances resulting in a vast distribution range over littoral areas of the tropics. A few species from divergent families are known in this plant group, and we categorized them into two groups based on the number of component species. One of them is "true-pantropical": *Ipomoea pes-caprae*

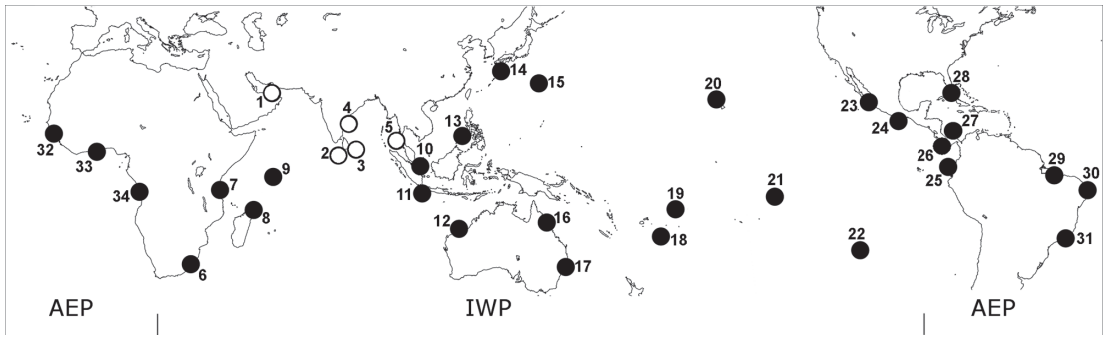

Fig. 1. Geographical distributions of the 34 populations used in this study. Each circle corresponds to one population that includes eight individuals. Populations of *Ipomoea pes-caprae* subsp. *pes-caprae* are shown by open circles, and subsp. *brasiliensis* by filled circles. Short vertical bars roughly suggests the positions of boundaries between IWP (Indo–West Pacific) and AEP (Atlantic–East Pacific) regions. Population numbers correspond to those in Table 1.

(L.) R. Br. (*Convolvulaceae*) and *Canavalia rosea* (Sw.) DC. (*Fabaceae*) are in this category. The other one is "sub-pantropical" species in which two or more closely related species are distributed in specific ranges but pantropical in total. Members of this category are some pairs or groups of plants: *Hibiscus tiliaceus* L. and *H. pernambucensis* Arruda (*Malvaceae*); *Vigna marina* (Burm.) Merr. and *V. luteola* (Jacq.) Benth. (*Fabaceae*); *Entada phaseoloides* (L.) Merr., *E. rheedei* Spreng. and *E. gigas* (L.) Fawc. & Rendle; and *Rhizophora* species (*Rhizophoraceae*).

One of the most successful studies of Pantropical plants with sea-drifted seeds by molecular markers were performed for *Hibiscus tiliaceus* and *H. pernambucensis*. Takayama et al. (2006 and 2008) used chloroplast and nuclear markers to study the history and levels of population differentiation, and revealed that, 1) substantial gene flow was retained across the Pacific to Indian Ocean, 2) American continents played a strong role as barriers and 3) historical migration and introgression occurred over the Atlantic. This research implied that the East Pacific and Atlantic oceans were so big barriers that the same species could not distribute across the barriers. However, it has not been studied yet whether those barriers were also effectively working within species and promoting population differentiation or speciation. Because none of the sub-pantropical species are distributed over those barriers, studying true-pantropical species only can answer to the question.

In this study, we used two low copy nuclear genes to reveal geographic structure of a true-pantropical species with sea-drifted seeds, *Ipomoea pes-caprae*. The species, commonly called beach morning glory, has pink, large flowers and sea-drifted seeds, and often forms large creeping mats on beaches and coastal dunes throughout tropical and subtropical regions of the world (St. John 1970, Devall 1992). The species comprises two subspecies, ssp. *pes-caprae* and ssp. *brasiliensis* (L.) Ooststr, which differ in the shape of the leaves and the dimensions of calyx and corolla, as well as in distribution ranges: ssp. *pes-caprae* is limited to the northern part of Indian Ocean (from Arabia to Indonesia) and ssp. *brasiliensis* is pantropical (St. John 1970, Fosberg and Sachet 1977, Fig. 1). A preliminary study using chloroplast sequences of intergenic spacer (IGS) for more than 4 kbp showed no molecular differences neither between the two subspecies nor among populations of ssp. *brasiliensis* (Tadashi Kajita unpublished). As no variation was obtained in cpDNA markers, more neutral molecular markers with higher substitution rates are

required. In this study, we employed two low copy nuclear genes, *HSP-90* and *Waxy*, to study the geographic structure of the species by investigating the distribution of haplotypes. The goal of the study is to reveal global geographic structure in a true-pantropical species, *Ipomoea pes-caprae*, and understand how the structure was formed.

Materials and Methods

Population sampling and DNA extraction

Leaf samples of 272 individuals from 34 populations of *Ipomoea pes-caprae* were collected over the distribution range of the species (Table 1, Fig. 1). Five of these populations (40 individuals; Nos. 1–5 of Table 1 and Fig. 1) were for *Ipomoea pes-caprae* subsp. *pes-caprae* which were collected from northern part of Indian Oceanic region. The rest of them were from *I. pes-caprae* subsp. *brasiliensis* (Nos. 6–34). Leaf fragment samples were collected in fields and kept and dried in silica-gel. Voucher specimens of these samples were deposited in the Herbarium of the University of the Ryukyus (URO). Total DNA was isolated from the silica-gel dried leaf samples using the cetyltrimethyl ammonium bromide (CTAB) extraction method as was described in a previous study (Doyle & Doyle 1987), with the addition of purifying the DNA solution with Glass Milk powder using GeneClean III kit (Bio 101, Vista, CA).

Locus selection, Polymerase Chain Reaction (PCR) and Sequencing

Two low copy genes, heat shock protein 90 (*HSP-90*) (Steele et al. 2008) and *Waxy* gene encoding granule-bound starch synthase I (GBSSI) (Miller et al. 1999) used for phylogenetic studies of *Ipomoea* were selected as genetic markers. We first confirmed whether clear sequence data could be obtained for these markers by direct PCR sequencing method using the primers published in the previous studies. The primers used for *HSP-90* were Steel 15-F(ACG GAC AAG AGC AAG CTC GAT G) and Steel 15-R(TTG TAG TCT TCC TTG TTC TCA G) (Steele et al. 2008), and for *Waxy* F(GAT ACC CAA GAG TGG AAC CC) and R(GTT CCA TAC GCA TAG CAT G) (Miller et al. 1999). We obtained clear single bands in PCR and clear sequence data by direct sequencing for both genes. We then designed internal specific primers for these two loci: for *HSP-90* (F: TAA CGT ATT ATG TAT TTG CAC TGA T, R: TTT AAT TAC AGA ACC AGA ATT ACC T) and for *Waxy* (F: GCT CAT TTG GAA TTT TAT GC, R: AAA TCA GCA CCA GCA GTA AT) based on conserved regions in the alignment including *I. pes-caprae* as well as all possible relevant sequences from closely related *Ipomoea* species obtained from GenBank. The partial sequence of these genes included both coding and noncoding regions (Table 2). Direct PCR sequencing using the same primers as sequencing primer was performed as was described in Vatanparast et al. (2011), using the ABI 3500 automated sequencer (Applied Biosystems, Foster City, CA).

All of the samples for the two markers produced clear sequence profiles. Although double peaks would be expected for possible heterozygotes in each marker, all the overlapping peaks detected in this study were from only one site. This allowed us to extract the two allelic nucleotide sequences when we detected a double peak. All the obtained sequences were visually determined and aligned by SEQSCAPE V2.5 software (Applied Biosystems). All sequence data were deposited in GenBank with accession nos. KF296518-24 and KF296558-62.

Nucleotide diversity and neutrality tests

Sequences were aligned and edited using BioEdit v. 5.0.9.1 (Hall 1999). Coding regions and open reading frames were identified by comparison with the *Ipomoea batatas* EST data from GenBank. For each locus and for each pair of populations, nucleotide diversity in terms of π (Nei and Li 1979) at total sites (πt), silent sites (πs) and nonsynonymous sites (πa) were estimated using DnaSP v. 5.10.01 (Librado

Table 1. Samples of *Ipomoea pes-caprae* used in this study

No.	Subspecies/region/locality	Country	N	Latitude	Longitude
	subsp. ***pes-caprae*** Indian Ocean				
1	Fujaira	UAE	8	25.493	56.361
2	Wattala, Negambo	Sri Lanka	8	6.997	79.873
3	Hambantoa	Sri Lanka	8	6.135	81.135
4	Maharashutra	India	8		
5	Kmala Beach, Phuket	Thailand	8	7.807	98.299
	subsp. ***brasiliensis*** IWP				
6	Tegula river mouth	South Africa	8	−29.23	31.487
7	Mjimwema	Tanzania	8	−6.836	39.323
8	Sambava	Madagascar	8	−14.295	50.189
9	Anse A La Manche, Mahe I.	Seychelles	8	−4.961	55.592
10	Southwest of Changi Airport	Singapore	8	1.317	103.981
11	Pantai Tratae, Java	Indonesia	8	−6.989	106.381
12	Fucarne I., Port Headland, West Australia	Australia	8	−20.307	118.584
13	Tak Doan, Puerto Princesa, Palawan I.	Philippines	8	9.461	118.587
14	Takanabe, Miyazaki Pref.	Japan	8	32.161	131.534
15	Ogasawara I., Tokyo Pref.	Japan	8	26.609	142.179
16	N. of Ellis Beach, Queensland	Australia	8	−16.717	145.635
17	Crowdy Head, Harrington, New South Wales:	Australia	8	−31.849	152.748
18	Haashini-Lavengatonga	Tonga	8	−21.12	−175.229
19	NW seashore, Upolu I.	Samoa	8	−13.863	−171.694
20	Oahu I., Hawaii	USA	8	21.367	−157.711
21	Anaho, Nuku Hiva, Marquesus I.	French Polynesia	8	−9.951	−139.049
22	Tongaliki, Easter I.	Chile	8	−27.122	−109.175
	AEP				
23	Playa Majahual, Sinaloa	Mexico	8	22.797	−105.959
24	Loca Blanca, Oaxaca	Mexico	8	15.709	−96.718
25	Esmeralda	Ecuador	8	0.958	−79.709
26	Vera Cruz	Panama	8	8.915	−79.568
27	Cuango, Colon	Panama	8	9.392	−79.871
28	Indian River, Florida	USA	8	27.872	−82.851
29	Praia do Crispin, Para	Brazil	8	−0.647	−47.562
30	Barra de Sirinhaem, Pernanbuco	Brazil	8	−8.618	−35.06
31	Recreio, Rio de Janeiro	Brazil	8	−22.996	−43.325
32	Joal-Fadiout	Senegal	8	14.256	−16.904
33	Labadi Beach	Ghana	8	5.583	−0.106
34	Musul, Luanda	Angola	8	−8.856	13.199

and Rozas 2009). To confirm that the variation obtained for the two markers were neutral, departures from neutral expectation were tested using Tajima's D (Tajima 1989), Fu and Li's D*(Fu and Li 1993) by DnaSP (Librado and Rozas 2009).

Phylogenetic and network analyses

Phylogenetic analyses were performed using the Wagner maximum parsimony (MP) method in PAUP* version 4.0 beta10 (Swofford 2002) as was described in Vatanparast et al. (2011). Bootstrap values were calculated based on 10,000 replicates searched by branch and bound algorithm. We used sequence data of *HSP-90* of *Ipomoea nil* and *Waxy* of *Ipomoea argillicola* obtained from GenBank as outgroups, according to the phylogenetic tree including *I. pes-caprae* and those species (Miller et al. 1999). To show the relationships among haplotypes, a statistical

Table 2. Nucleotide variations of *HSP-90* and *Waxy* genes of *Ipomoea pes-caprae*

	length (bp)	exon	intron	H	Hd	πt	πs	πa	Tajima's D	Fu & Li D*
HSP-90	785	1–41, 481–785	40–481	7	0.77	0.0026	0.0098	0.0012	0.590	1.041
Waxy	574	1–46, 126–301, 405–574	47–125, 302–404	5	0.58	0.0016	0.0065	0.0011	0.862	1.555

Exon and intron suggest presumed positions in the alignment.
Nucleotide diversities are shown as of all sites (πt), of synonymous sites (πs) and of non-synonymous sites (πa). See text for detail. H. Number of haplotypes. Hd. Haplotype diversity.

parsimony network was inferred for each locus using TCS1.06 (Clement et al. 2000).

Results

Nucleotide variation and neutrality tests

Two nuclear loci were sequenced for 272 individuals representing 34 populations of the species. The aligned length of nucleotide sequence was 785 bp for *HSP-90* and 574 for W*axy* (Table 2). The level of polymorphism was compared between the two loci by nucleotide diversity, and they are in similar level, but *HSP-90* was a little more polymorphic (Hd = 0.77 and πt = 0.0026) than *Waxy* (Hd = 0.58 and πt = 0.0016) (Table 2). The diversity at noncoding and synonymous sites was higher than that at nonsynonymous sites. In neutrality tests, both genes showed no significant departure from neutrality in Tajima's D and Fu and Li's D*(Table 2).

Phylogenetic relationships

The total number of haplotypes recognized in this study was seven (A1–A7) for *HSP*-90 and five (B1–B5) for *Waxy* (Table 3, Fig. 2). Phylogenetic analyses of haplotypes including outgroups inferred only one most parsimonious tree (tree length = 6, consistency index (CI) = 1.0 and retention index (RI) = 1.0) for *HSP-90*, and two trees (tree length = 9, CI = 0.89, RI = 0.67) for *Waxy* (Fig. 2). In the *HSP-90* tree, two clades with bootstrap of 63 % were recognized, one of which consisted of A4 (orange in Fig. 2a) and A5 (blue), and the other of A6 (green) and A7 (red). In the majority rule consensus tree of *Waxy* (Fig. 2d), B2 (green) and B4 (blue) formed a clade with bootstrap value 69, which is sister to B3 (yellow) with bootstrap support less than 50 %.

Haplotype Network

In the haplotype network of *HSP-90* (Fig. 2b), A7 (red) that is the dominant haplotype of ssp. *pes-caprae* was connected with one step to A6 (green). Other minor haplotypes, A1 (purple), A3 (yellow) and A5 (blue), which appeared in less than 10 samples, were connected with one step to major haplotypes A4 (orange) or A2 (black), which appeared in more than 10 samples. In the haplotype network of *Waxy* (Fig. 2e), minor haplotype B3 (yellow in Fig. 2d) was connected with one step to a major haplotype B1 (orange). A major haplotype B5 (black) was also connected with one step to B1 (orange).

Geographic distribution of haplotypes

The geographic distribution of haplotypes were shown on maps (Fig. 2). In the *HSP-90* map (Fig. 2c), three major haplotypes, A2 (black in Fig. 2c), A4 (orange) and A6 (green), had wide distributions across more than one geographic region. A6 (green) was appeared in both ssp. *pes-caprae* and ssp. *brasiliensis*. A2 (black) was the only haplotype that appeared in the American Continents, and was also in the Pacific, Atlantic and Indian Oceanic regions. Both A4 (orange) and A6 (green) appeared widely from Africa to the Pacific regions. A7 (red) was the dominant haplotype of ssp. *pes-caprae*, and only appeared in the northern part

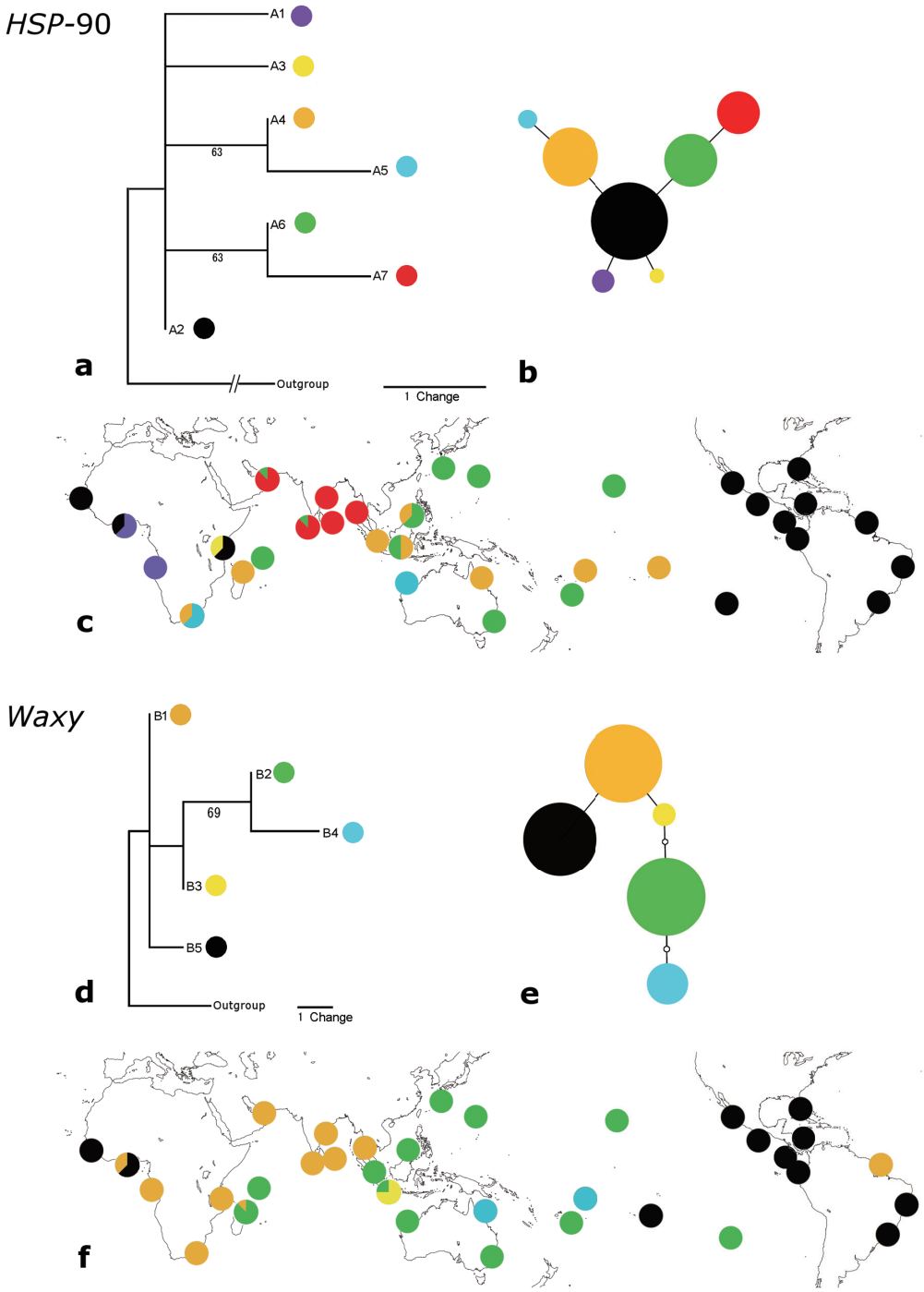

Fig. 2. Phylogenetic trees, network and geographic distribution of haplotypes obtained from *HSP-90* (a–c) and *Waxy* (d–f) of *Ipomoea pes-caprae*. a, d. Maximum parsimony trees showing phylogenetic relationships among haplotypes (see details in text). In figures for each gene, each color designates the same haplotype. b, e. Haplotype network. Each haplotype is shown as a circle the size of which is proportional to the number of individuals possessing the haplotype. Mutational steps connecting haplotypes are represented by small open circles. e, f. Geographic distribution of haplotypes. Each circle is a pie chart for a population that includes eight individuals.

Table 3. Haplotype tables for *HSP-90* and *Waxy* genes of *Ipomoea pes-caprae*. Polymorphic sites are designated by positions from the 5' end in the alignment. Dot (•) indicates that the character state is same as the one in the first line.

HSP-90	Haplotype	92	255	272	295	510	683	778
	A1	T	-	G	C	A	G	T
	A2	.	T	.	T	.	.	.
	A3	G	T	.	T	.	.	.
	A4	.	T	.	T	.	A	.
	A5	.	T	.	T	T	A	.
	A6	.	T	.	T	.	.	C
	A7	.	T	C	T	.	.	C
Waxy		40	61	327	382	540		
	B1	C	G	G	A	G		
	B2	T	C	.	G	.		
	B3	T	.	.	.	.		
	B4	.	C	.	G	A		
	B5	.	.	A	.	.		

of the Indian Oceanic region. Other minor haplotypes, A1 (purple), A3 (yellow), and A5 (blue) appeared in the West Africa, in East Africa, and in East Africa and West Australia, respectively.

In the *Waxy* map (Fig. 2f), three major haplotypes, B1 (orange), B2 (green) and B5 (black), appeared across more than one geographic region. B1 (orange) was shared by both ssp. *pes-caprae* and ssp. *brasiliensis*. B1 (orange) appeared from the Atlantic America, West and East Africa to the Northern Part of Indian Ocean (as ssp. *pes-caprae*). B2 (green) appeared from the East Africa to South Pacific. B5 (black) appeared from the South Pacific, West and East side of American Continents and West Africa. Other minor haplotypes, B3 (yellow) and B4 (blue) appeared in the Eastern part of the Indian Oceanic region, and in Oceania to the South Pacific. The number of haplotypes appeared in geographical regions were summarized in Table 4.

Discussion

Studying the 272 samples of *Ipomoea pes-caprae* collected from 34 populations around the globe by two low copy nuclear genes *HSP-90* and *Waxy*, seven and five haplotypes respectively were detected. The level of polymorphism in the nuclear genes was much higher than cpDNA in which no variation was found in the analyses using more than 4,000 bp from a wide range of distribution (Tadashi Kajita, unpublished). The levels of polymorphism shown by nucleotide diversity of the markers (πt in Table 2) were 0.0026 for *HSP-90* and 0.0016 for *Waxy* which was not very different from the variations of nuclear markers studied in another sea-dispersed plants *Bruguiera gymnorhiza* in which average πt was 0.008 (Urashi et al. 2013). The variations of the two genes analyzed were not significantly departed from neutral, hence the two markers would be used to analyze the demographic history of *I. pes-caprae* by using distribution maps, haplotype trees and networks.

AEP-IWP like geographic structure with small migration over boundaries

The resultant distribution maps of haplotypes of both nuclear genes suggest that *Ipomoea pes-caprae* has a clear geographic structure: a few haplotypes (A2 and A1 of *HSP-90*; B5 and B1 of *Waxy*) appeared predominantly in the American Continents and West Africa, and the others appeared also predominantly from East Africa to the South Pacific across Indian Oceanic and Pacific regions (Fig. 2). The distribution pattern is well consistent with AEP (Atlantic and East

Table 4. Summary of number of haplotypes of *HSP-90* and *Waxy* in *Ipomoea pes-caprae* by regions. The column of "*pes-caprae*" designates the number of haplotypes obtained from *I. pes-caprae* subsp. *pes-caprae* and other columns from subsp. *brasiliensis*

	pes-caprae	Africa	Asia-Oceania	Pacific Island	America
HSP-90	2	6	3	3	1
Waxy	1	3	3	3	2
Total	3	9	6	6	3

	E A	W IO	E IO	W Pacific	East Pacific	W Atlantic
HSP-90	2	5	3	3	1	1
Waxy	2	2	3	3	1	2
Total	4	7	6	6	2	3

EA. East Atlantic. W IO. West Indian Ocean. E IO. East Indian Ocean

Pacific) and IWP (Indo-West Pacific) regions.

The AEP and IWP regions are biogeographic regions and the distribution pattern following them is a common feature of many sea-dispersed plants including mangroves (Triest 2008). The AEP region is over the area from the American Continents, Atlantic Ocean and East Africa, and the IWP region is the other area of the globe from East Africa to the South Pacific. Many sea-dispersed plant species are exclusively distributed in either region, and the boundaries of the distribution are at the African continent and the East Pacific Ocean. These land and oceanic barriers are likely to be very strong to prevent migration between the AEP and IWP regions for many sea-dispersed plants.

In our study, a true-pantropical species, *Ipomoea pes-caprae*, has genetic structure consistent with the biogeographic region AEP and IWP. This implies that the barriers have been strong enough to shape the genetic structure of the species. However, the barriers would not have been quite strong enough to prevent the gene flow between the regions, because there are a few common haplotypes distributed in both AEP and IWP regions (A2 of *HSP-90*, B1 of *Waxy* in two population of West Africa, and B5 of *Waxy* in a population of the South Pacific). These results will suggest that the sea-dispersal ability of *Ipomoea pes-caprae* would be sufficient to override the barriers and to keep gene flow over the barriers, even though population differentiation happened. Violations to the regular AEP-IWP distribution pattern were also observed in other two groups of sub-pantropical species, *Hibiscus tiliacues – H. pernambucensis* (Takayama et al. 2006, 2008) and *Rhizophora* species (Takayama et al. 2013).

Gene flow is kept across the extremely wide distribution by long distance dispersal of sea-drifted seeds

In spite of the genetic structure between IWP and AEP regions, most of the major haplotypes of *Ipomoea pes-caprae* were extremely widely distributed, which would be caused by dispersal by sea-drifted seeds. In the distribution maps (Fig. 2), major haplotypes except for A7 of *HSP-90* were distributed over two or more oceanic regions, with the widest distribution of A2 of *HSP-90* in the Atlantic, Pacific and Indian Oceanic regions. These extremely wide distributions of haplotypes overriding the two major barriers for other sea-dispersed plants suggests that this species has very effective long distance dispersal by sea-drifted seeds. Indeed, our preliminary study suggested that the seeds of *Ipomoea pes-caprae* could float on seawater for two years, retaining viability (Yoichi Tateishi unpublished data).

The relationship between the two subspecies

The results of this study may suggest that the origin of ssp. *pes-caprae* was from ssp.

brasiliensis. A major haplotype A7 of *HSP-90* specifically appeared in ssp. *pes-caprae* and it was monophyletic with A6 that is one of the major haplotypes of ssp. *brasiliensis* distributed in the Indian Oceanic and Pacific region and partially appeared in ssp. *pes-caprae* (Figs. 2a, 2c). *Waxy* gene also showed that the two subspecies shared the same haplotype B1, which may suggest the diversification of the two subspecies might not be very old. Subspecies *pes-caprae* might be diversified from ssp. *brasiliensis* at the northern margin of the distribution of ssp. *brasiliensis* in the northern part of the Indian Oceanic region. This kind of speciation at the edge of the wide distribution range of pantropical plants with sea-drifted seeds were also reported in *Hibiscus tiliaceus* and its allied species (Takayama et al. 2006).

Implication on the route of population expansion

The present distributions of haplotypes and phylogenetic trees as well as networks suggests the route of population expansion might be from Africa or Indian Oceanic region to the Pacific and Atlantic regions. The number of haplotypes appeared in geographical regions were summarized in Table 4 and showed the tendency that higher number of haplotype in Africa and Indian Oceanic regions and lower in the New World. In both genes, the New World populations are almost fixed to a single haplotype in each DNA region (A2 for *HSP-90* and B5 for *Waxy* in Fig. 2), which suggests relatively newer origin of the new world population from small founder populations that have experienced severe bottleneck, or the populations were under severe selection. In the haplotype network of *Waxy* (Fig. 2e), the new world haplotype B5 is closer to B1 (1 step) of the Atlantic and Indian Oceanic regions than to B2 (4 steps) in the Pacific and Indian Oceanic regions. This suggests an expansion from Africa or Indian Oceanic region both westward and eastward.

We need to pay special attention to the similar distribution patterns of haplotypes (A1 in *HSP-90* and B5 in *Waxy* in Fig. 2c, f) across the New World. Although our data suggest that the establishment of the New World population is newer, the Isthmus of Panama was closed about 3.1–3.5 million years ago (Knowlton et al. 1993, Marko 2002). Sea-drifted seeds could not be dispersed through the Isthmus of Panama after the closure. So, the presence of the same haplotypes in both Pacific and Atlantic sides of the American Continents would be established either by migration of seeds 1) across the Pacific, Indian and Atlantic Oceans or 2) through the Isthmus of Panama by other vectors like animal or human. We still don't have enough data to answer which case is more plausible, but further analyses increasing the number of markers and samples may answer this question. Gene flow between the Pacific and Atlantic side populations were also reported using microsatellite markers in other sub-pantropical species, *Hibiscus pernambucensis* (Takayama et al. 2008) and *Rhizophora mangle* (Takayama et al. 2013).

Conclusion

This is the first phylogeographic study on a true pantropical plant with sea-drifted seeds, *Ipomoea pes-caprae*. Our data using two nuclear genes showed significant genetic differentiation between the IWP and AEP regions, and distribution of the same haplotypes throughout the two regions. These results suggest that dispersal by sea-drifted seeds was responsible for gene flow across the wide distribution range, and that both the East Pacific and African Continent acted as geographical barriers for the migration by drifted seeds even for the true pantropical plants. Presence of more haplotypes in Africa and Indian Oceanic region may imply that the origin of the species was in these regions, and that the species expanded its distribution to the New World.

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References

Clement M., Posada D. and Crandall K. A. 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. **9**: 1657–1659.

Devall M. S. 1992. The biological flora of coastal dunes and wetlands. 2. *Ipomoea pes-caprae* (L.) Roth. J. Coastal. Res. **8**: 442–456.

Doyle J. and Doyle J. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemistry **19**: 11–15.

Fosberg F. R. and Sachet M. H. 1977. Flora of Micronesia. 3. *Convolvulaceae*. Smithsonian Contrib. Bot. **36**: 1–34.

Fu Y.-X. and Li W.-H. 1993. Statistical tests of neutrality of mutations. Genetics **133**: 693–709.

Hall T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. **41**: 95–98.

Knowlton N., Weigt L. A., Solórzano L. A., Mills D. K. and Bermingham E. 1993. Divergence in proteins, mitochondrial DNA, and reproductive compatibility across the Isthmus of Panama. Science **260**: 1629–1632.

Librado P. and Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics **25**: 1451–1452.

Marko P. B. 2002. Fossil calibration of molecular clocks and the divergence times of geminate species pairs separated by the Isthmus of Panama. Mol. Biol. Evol. **19**: 2005–2021.

Miller R. E., Rausher M. D. and Manos P. S. 1999. Phylogenetic systematics of *Ipomoea* (*Convolvulaceae*) based on ITS and waxy sequences. Syst. Bot. **24**: 209–227.

Nei M. and Li W.-H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. **76**: 5269–5273.

Steele P. R., Guisinger-Bellian M., Linder C. R. and Jansen R. K. 2008. Phylogenetic utility of 141 low-copy nuclear regions in taxa at different taxonomic levels in two distantly related families of rosids. Mol. Phylogenet. Evol. **48**: 1013–1026.

St. John H. 1970. Classification and distribution of the *Ipomoea pes-caprae* group (*Convolvulaceae*). Bot. Jahrb. **89**: 563–583.

Swofford D. 2002. PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4. Sinauer Associates, Sunderland.

Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics **123**: 585–595.

Takayama K., Kajita T., Murata J. and Tateishi Y. 2006. Phylogeography and genetic structure of *Hibiscus tiliaceus*— speciation of a pantropical plant with sea-drifted seeds. Mol. Ecol. **15**: 2871–2881.

Takayama K., Tateichi Y., Murata J. and Kajita T. 2008. Gene flow and population subdivision in a pantropical plant with sea-drifted seeds *Hibiscus tiliaceus* and its allied species: evidence from microsatellite analyses. Mol. Ecol. **17**: 2730–2742.

Takayama K., Tamura M., Tateishi Y., Webb E. and Kajita T. 2013. Strong genetic structure over the American continents and transoceanic dispersal in the mangrove genus *Rhizophora* (*Rhizophoraceae*) revealed by broad-scale nuclear and chloroplast DNA analysis. Amer. J. Bot. **100**: 1191–1201.

Triest L. 2008. Molecular ecology and biogeography of mangrove trees towards conceptual insights on gene flow and barriers: A review. Aquat. Bot. **89**: 138–154.

Urashi C., Teshima K. M., Minobe S., Koizumi O. and Inomata N. 2013. Inferences of evolutionary history of a widely distributed mangrove species, *Bruguiera gymnorrhiza*, in the Indo-West Pacific region. Ecol. Evol. **3**: 2251–2261.

Vatanparast M., Takayama K., Sousa M. S., Tateishi Y. and Kajita T. 2011. Origin of Hawaiian endemic species of *Canavalia* (*Fabaceae*) from sea-dispersed species revealed by chloroplast and nuclear DNA sequences. J. Jpn. Bot. **81**: 15–25.

M. Miryeganeh[a]，高山浩司[b]，立石庸一[c]，梶田　忠[a]：
核マーカーによって示された汎熱帯海流散布植物グンバイヒルガオ（ヒルガオ科）の地理的構造

汎熱帯海流散布植物とは，熱帯域を中心に地球を一周するほど広い分布域を持つ，海流散布植物の総称である．その構成要素は，グンバイヒルガオやナガミハマナタマメ等，1 種のみで汎熱帯域に広がる分布域を持つ汎熱帯種と，オオハマボウとアメリカハマボウ，あるいはハマアズキとナガバハマササゲ等，互いにごく近縁ないくつかの種の分布域を合わせると汎熱帯域になる準汎熱帯種の，2 つのグループに分けられる．本研究では，汎熱帯海流散布植物のうち，代表的な汎熱帯種であるグンバイヒルガオ *Ipomoea pes-caprae*（ヒルガオ科）が，広大な分布域内でどのような遺伝構造を持つかを，2 つの核遺伝子マーカー（*HSP-90* と *Waxy*）を用いて明らかにした．分布域を広く網羅するような地域から採集された 34 集団 272 個体で 2 つの遺伝子の塩基配列を決定し，系統解析，ネットワーク解析，ハプロタイプ分布の比較を行ったところ，IWP（インド洋―西太平洋地域）と AEP（大西洋―東太平洋地域）にそれぞれ特有のハプロタイプが分布する，明瞭な遺伝構造の存在が明らかになった．このことは，アフリカ大陸と東太平洋が，IWP と AEP の間の種子の移動を妨げていることを示唆している．一方，それぞれの地域内では，海で遠く隔てられた集団間でも同一のハプロタイプが見られることから，海流による長距離種子散布が，広大な分布域内における遺伝子流動の維持に貢献していることが示唆された．また，ハプロタイプの数を地域ごとに比較したところ，アフリカ大陸とインド洋地域で多く，新大陸ではほぼ 1 つに固定しており，アフリカ大陸かインド洋地域から新大陸への移動が生じたことが示唆された．一方，新大陸東西の集団間でも共通のハプロタイプが分布していることから，パナマ地峡を越えるような遺伝子流動が起こった可能性も示唆された．

（[a] 千葉大学大学院理学研究科生物学コース，
[b] 東京大学総合研究博物館，
[c] 琉球大学教育学部）