



Genetic structure of *Ipomoea imperati* (Convolvulaceae) in the Mediterranean region and implications for its conservation

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Abstract

In this paper, we studied the relationships of the only surviving Italian population of *Ipomoea imperati* (Convolvulaceae), a pantropical sandy coastal species, in Sicily and other populations in the Mediterranean region. Herbarium samples which are representative of extinct populations growing in Campania (Italy) were also investigated together with populations from various Atlantic and Mediterranean localities. Chloroplast DNA microsatellites (cp-SSR) and nuclear ribosomal Internal Transcribed Spacer (ITS) sequences were jointly employed, in order to detect relationships among populations. Our aims were several-fold: (1) to clarify if the species is autochthonous in the Mediterranean basin or a post-Columbian introduction; (2) to investigate phylogeographic patterns in the species and (3) to establish the possible role of dispersal in explaining the patterns observed. Chloroplast microsatellite variation indicates that extinct Italian mainland populations of *I. imperati* from Campania are not closely related to the extant Sicilian one, as they do not share haplotypes. Chloroplast DNA microsatellite variation is largely between populations, and the within populations component accounts for only approximately 10%. CpDNA data is consistent with a single Mediterranean entry point hypothesis or with the notion that some populations display plesiomorphic variability. ITS data is congruent with the possibility that the presence of *I. imperati* in the Mediterranean is the result of transatlantic dispersal. The population from Sicily and extinct populations from Campania share an ITS type. A Bayesian analysis employing clock calibration data on an expanded ITS dataset with appropriate outgroups indicates that dates of transoceanic distribution are probably earlier than historical times.

Key words: *Ipomoea imperati*, chloroplast DNA microsatellites, ITS sequences, Mediterranean region

Introduction

Ipomoea imperati (Vahl 1790:17) Grisebach (1866:203) (Convolvulaceae) is a pioneer coastal strand species of tropical and subtropical coastal regions. It is common in dune systems, where it is regarded as a dominant species, as it rapidly produces stolons and traps shifting sand (Judd *et al.* 1977, Judd & Sides 1983).

Ipomoea imperati is generally considered native of tropical Central America and of part of southeastern North America (Texas and Florida) (Lonard & Judd 1999; Silvestre 2012). However, it has a pantropical distribution, being present also in Asia, Pacific Islands and Australia, as well as in the Canary Islands, the Azores and the Mediterranean (Lonard & Judd 1999; Fang & Staples 1995, Austin *et al.* 2001), where the question of its origin is considered still open (McDonald 1991, Silvestre 2012, USDA 2012). In the Mediterranean basin, *I. imperati* is infrequently recorded for the Iberian peninsula (also in Balearic islands),

Our ITS sequence results suggest that anthropogenic dispersal is not involved in the presence of *I. imperati* in the Mediterranean, which, therefore is not related to exchanges between the Europe and the Americas in the period after Columbus' voyages. Our conclusion is at odds with the opinion of Silvestre (2012), who considered the species to be of recent introduction in Spain (the author excludes it altogether from the Balearic Islands).

Even if chloroplast microsatellite data would support one single transatlantic event, whereas ITS data would suggest that the event occurred more than once, both datasets indicate that long range dispersal is likely involved. We do not attempt here to reconcile the evidence obtained from chloroplast DNA with that from nuclear DNA; an expanded data set, with large samples from the extramediterranean locations here represented by single sequences and from the Asian part of the range which was not taken into account here will be needed to accomplish such a task. At present, however, the hypothesis that occasional, remote hybridisation events between individuals at distant sites (e.g., Sicily and Israel), followed by severe contraction of populations may have influenced the pattern indicated by chloroplast microsatellites (Figure 2) cannot be ruled out.

Protection of the Sicilian locality and reintroduction into the Naples/Ischia area appear appropriate. However, the habitat *I. imperati* (i.e., sandy coasts) is one of the most prone to human impact and severe degradation. Human activities in coastal areas, often lead to massive urbanization, including the building of large infrastructures/ industrial facilities, as well as to expanded tourism. A further disturbance is the steady erosion of the coastline in addition to changes in the morphology of the coastlines. These disturbances may cause, in extreme conditions, the almost total disappearance of the vegetation. In the case of the Neapolitan coast (Bagnoli), the construction of new buildings was directly responsible for the disappearance of *I. imperati* from the location (the only one recorded in the Italian mainland) already by the end of 19th century (Migliorato 1896, sub *Convolvulus imperati*). As far as Ischia island is concerned, the expansion of tourism, in the form of lidos, has deeply transformed the coast and caused the local extinction of the plant (Ricciardi *et al.* 2004). Restoration of these areas will certainly involve the reintroduction of plant species such as *I. imperati*, which were once are spontaneous in these coastal environments. In the absence of specimens having chloroplast haplotypes identical to those formerly present in Campania, our research suggests that the reintroduction of *I. imperati* into the Neapolitan coast, should be carried out using individuals from Sicily, which have identical ITS sequences.

References

- Aceto, S., Caputo, P., Cozzolino, S., Gaudio, L. & Moretti, A. (1999) Phylogeny and evolution of *Orchis* and allied genera based on ITS DNA variation: morphological gaps and molecular continuity. *Molecular Phylogenetics and Evolution* 13: 67–76.
<http://dx.doi.org/10.1006/mpev.1999.0628>
- Akaike, H. (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19: 716–723.
<http://dx.doi.org/10.1109/tac.1974.1100705>
- Austin, D.F., Kitajima, K., Yoneda, Y. & Qian, L. (2001) A putative American plant, *Ipomoea nil* (Convolvulaceae) in pre-Columbian Japanese art. *Economic Botany* 55: 515–527.
<http://dx.doi.org/10.1007/bf02871714>
- Bandelt, H.J., Forster, P. & Röhl, A. (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16: 37–48.
<http://dx.doi.org/10.1093/oxfordjournals.molbev.a026036>
- Bell, C.D., Soltis, D.E. & Soltis, P.E. (2010) The age and diversification of the angiosperms re-revisited. *American Journal of Botany* 97: 1296–1303.
<http://dx.doi.org/10.3732/ajb.0900346>
- Bremer, K. (1994) Branch support and tree stability. *Cladistics* 10: 295–304.
<http://dx.doi.org/10.1111/j.1096-0031.1994.tb00179.x>
- Brown, R. (1810) *Prodromus floræ Novæ Hollandiæ et Insulæ Van-Diemen : exhibens characteres plantarum quas annis*

- 1802-1805. Typis R. Taylor et socii, London, 590 pp.
- Brown, R. (1818) *Narrative of an expedition to explore the river Zaire, usually called the Congo, in South Africa, in 1816, under the direction of Captain J. K. Tuckey, R.N. To which is added, The journal of Professor Smith.* J. Murray, London, 498 pp.
- Coates, G., Jackson, J.B.C., Collins, L.S., Cronin, T.M., Dowsett, J., Bybell, L.M., Jorge, P.J. & Recope, A.O. (1992) Closure of the isthmus of Panama: the near-shore marine record of Costa Rica and western Panama. *Geological Society of America Bulletin* 104: 814–828.
[http://dx.doi.org/10.1130/0016-7606\(1992\)104%3C0814:cotiop%3E2.3.co;2](http://dx.doi.org/10.1130/0016-7606(1992)104%3C0814:cotiop%3E2.3.co;2)
- Conti, F., Abbate, G., Alessandrini, A. & Blasi, C. (Eds) (2005) *An annotated Checklist of the Italian Vascular Flora.* Palombi Editori, Roma, 420 pp.
- Dalechamps, J. (1586) *Historia generalis Plantarum.* Lyon, 1940 pp.
- Doyle, J.J. & Doyle, J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.
- Drummond, A.J., Ho, S.Y.W., Phillips, M.J. & Rambaut, A. (2006) Relaxed phylogenetics and dating with confidence. *PLOS Biology* 4: 699–71.
<http://dx.doi.org/10.1371/journal.pbio.0040088>
- Drummond, A. J., Suchard, M.A., Xie, D., & Rambaut, A. (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* 29: 1969–1973.
<http://dx.doi.org/10.1093/molbev/mss075>
- Excoffier, L., Smouse, P.E. & Quattro, J.M. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131: 479–491.
- Excoffier, L., Laval, G. & Schneider, S. (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1: 47–50.
- Fang, R. & Staples, G. (1995) In *Flora of China*. Wu ZY and Raven PT, 16: 271–325.
- Felsenstein, J. (1985) *PHYLIP (Phylogeny Inference Package) version 3.52c.* Distributed by the author. Department of Genetics, University of Washington, Seattle.
- Friis, E.M., Crane, P.R. & Pedersen, K.R. (2011) *Early Flowers and Angiosperm.* Cambridge University Press, Cambridge, 600pp.
- Gmelin, J.F. (1791) *Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, loci. Tomus II. Editio decimal tertia, aucta, reformata.* Impensis Georg. Emanuel. Beer, Lipsiae, 884pp.
- Goldstein, D.G. & Pollock, D.D. (1997) Launching microsatellites: a review of mutation processes and methods of phylogenetic inference. *The Journal of Heredity* 88: 335–342.
<http://dx.doi.org/10.1093/oxfordjournals.jhered.a023114>
- Goloboff, P. (1999) *Nona. Instruction manual.* Published by the author, S. M. de Tucumán, Argentina.
- Gray, A. (1862) *Characters of New or Obscure Species of Plants of Monopetalous Orders in the Collection of the United States South Pacific Exploring Expedition under Captain Charles Wilkes, U.S.N. With occasional Remarks, & c.* Proceedings of the American Academy of Arts and Sciences 5: 321–352.
<http://dx.doi.org/10.2307/20021271>
- Greuter, W., Burdet, H.M. & Long, G. (1986) *Med-Checklist: A critical inventory of vascular plants of the circum-Mediterranean countries, 3. Dicotyledones (Convolvulaceae-Labiatae).* Conservatoire et Jardin Botanique, Ville de Genève, Genève & Berlin, 395pp.
- Grisebach, A. (1866) *Catalogus plantarum cubensium.* Lipsiae, 309 pp.
- Gunn, C.R. & Dennis, J.V. (1976) *World guide to tropical Drift Seeds and Fruits.* Fitzhenry and Whiteside, Toronto, 240 pp.
- Guppy, H.B. (1917) *Plants, seeds and currents in the West Indies and Azores.* William and Norgate, London, 531 pp.
- Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Hallier, H. (1893) Versuch einer natürlichen Gliederung der Convolvulaceen auf morphologischer und anatomischer Grundlage. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 16: 453–591.
- House, H.D. (1921) Necessary changes of certain plant names. *New York State Museum Bulletin* 223: 60–69.
- Huttunen, S., Hedenäs, L., Ignatov, M.S., Devos, N. & Vanderpoorten, A. (2008) Origin and evolution of the northern hemisphere disjunction in the moss genus *Homalothecium* (Brachytheciaceae). *American Journal of Botany* 95: 720–730.
<http://dx.doi.org/10.3732/ajb.2007407>
- Imperato, F. (1599) *Historia naturale. Libro vigesimo ottavo.* Napoli, 791pp.
- Imperato, F. (1672) *Historia naturale. Libro vigesimo ottavo, 2.* Giovanni Maria Ferro, Venezia, 696 pp.
- Ishii, T., Takahashi, C., Ikeda, N., Kamijima, O. & Mori, N. (2006) Mitochondrial microsatellite variability in common wheat and its ancestral species. *Genes and Genetic Systems* 81: 211–214.

- Jarne, P. & Lagoda, P.J.L. (1996) Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution* 11: 424–430.
[http://dx.doi.org/10.1016/0169-5347\(96\)10049-5](http://dx.doi.org/10.1016/0169-5347(96)10049-5)
- Judd, F.W., Lonard, R.I. & Sides, S.L. (1977) The vegetation of South Padre Island, Texas, in relation to Topography. *Southwestern Naturalist* 22: 31–48.
<http://dx.doi.org/10.2307/3670462>
- Judd, F.W. & Sides, S.L. (1983) The effect of Hurricane Allen on the nearshore vegetation of South Padre Island. *Southwestern Naturalist* 28: 365–369.
<http://dx.doi.org/10.2307/3670799>
- Käss, E. & Wink, M. (1997) Phylogenetic relationships of the *Papilionoideae* (Family Leguminosae) based on nucleotide sequences of cpDNA (rbcL) and ncDNA (ITS 1 and 2). *Molecular Phylogenetics and Evolution* 8: 65–88.
<http://dx.doi.org/10.1006/mpev.1997.0410>
- Kay, K.M., Whittall J.B. & Hodges S.A. (2006) A survey of nuclear ribosomal internal transcribed spacer substitution rates across angiosperms: an approximate molecular clock with life history effects. *BMC Evolutionary Biology* 6: 36.
- Knevel, I.C. (2001) *The life history of selected coastal for e dune species of South Africa*. PhD Thesis Rhodes University, Grahamstown, Z.A.
- Koch, M.A., Dobes, C. & Mitchell-Olds, T. (2003) Multiple hybrid formation in natural populations: concerted evolution of the Internal Transcribed Spacer of nuclear ribosomal DNA (ITS) in North American *Arabis divaricarpa* (Brassicaceae). *Molecular Biology and Evolution* 20: 338–350.
<http://dx.doi.org/10.1093/molbev/msg046>
- Koga, K., Kadono, Y. & Setoguchi, H. (2008) Phylogeography of Japanese water crowfoot based on chloroplast DNA haplotypes. *Aquatic Botany* 89: 1–8.
<http://dx.doi.org/10.1016/j.aquabot.2007.12.012>
- La Valva, V. & Sabato, S. (1983) Nomenclature and typification of *Ipomoea imperati* (Convolvulaceae). *Taxon* 32: 110–132.
- Levinson, G. & Gutman, G.A. (1987) Slipped-strand mispairing: a major mechanism for DNA sequence evolution. *Molecular Biology and Evolution* 4: 203–221.
- Linnaeus, C. (1753) *Species plantarum*. Impensis Laurentii Salvii, Stockholm, 1200 pp.
- Lonard, I. & Judd, W.F. (1999) The biological flora of coastal dunes and wetlands. *Ipomoea imperati* (Vahl) Griseb. *Journal of Coastal Research* 15(3): 645–652.
- Mantel, N. (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research* 27: 209–220.
- McDonald, A. (1991) Origin and diversity of Mexican Convolvulaceae. *Anales del Instituto de Biología de la Universidad Nacional Autónoma de México, Serie Botánica* 62: 65–82.
- Migliorato, E. (1896) Osservazioni relative alla flora napoletana. *Bollettino della Società Botanica Italiana*. 7: 168–171.
- Miller, K.G. & Fairbanks, R.G. (1983) Evidence for Oligocene-Middle Miocene abyssal circulation changes in the western North Atlantic. *Nature* 306: 250–253.
<http://dx.doi.org/10.1038/306250a0>
- Miller, R.E. & Rausher, M.D. (1999) Phylogenetic Systematics of *Ipomoea* (Convolvulaceae) Based on ITS and Waxy Sequences. *Systematic Botany* 24: 209–227.
<http://dx.doi.org/10.2307/2419549>
- Millsbaugh, C.F. (1900) *Plantae utowanae*. Field Columbian Museum. *Botanical series* 2(1): 3–110.
- Milne, R.I. & Abbott, R.J. (2002) The origin and evolution of Tertiary relict floras. *Advances in Botanical Research* 38: 281–314.
[http://dx.doi.org/10.1016/s0065-2296\(02\)38033-9](http://dx.doi.org/10.1016/s0065-2296(02)38033-9)
- Muir, G., Fleming, C.C. & Schlötterer, C. (2001) Three divergent rDNA clusters predate the species divergence in *Quercus petraea* (Matt.) Liebl. and *Quercus robur* L. *Molecular Biology and Evolution* 18: 112–119.
<http://dx.doi.org/10.1093/oxfordjournals.molbev.a003785>
- Muir, G. & Schlötterer, C. (2005) Evidence for shared ancestral polymorphism rather than recurrent gene flow at microsatellite loci differentiating two hybridizing oaks (*Quercus* spp.). *Molecular Ecology* 14: 549–61.
<http://dx.doi.org/10.1111/j.1365-294x.2004.02418.x>
- Muller, J. (1981) Fossil pollen records of extant angiosperms. *Botanical Review* 47: 1–142.
<http://dx.doi.org/10.1007/bf02860537>
- Namoff, S., Luke, Q., Jimenez, E.F., Veloz, A., Lewis, C.L., Sosa, V., Maunder, M. & Ortega, J.F. (2010) Phylogenetic analyses of nucleotide sequences confirm a unique plant intercontinental disjunction between tropical Africa, the Caribbean, and the Hawaiian Islands. *Journal of Plant Research* 123: 57–65.
<http://dx.doi.org/10.1007/s10265-009-0258-0>

- Nixon, K.C. (1999) *Winclada (beta) ver. 0.9.9*. Published by the author, Ithaca, New York.
- Paape, T., Igic, B., Smith, S.D., Olmstead, R., Bohs, L. & Kohn, J.R. (2008) A 15 Myr-Old Genetic Bottleneck. *Molecular Biology and Evolution* 25: 655–663.
<http://dx.doi.org/10.1093/molbev/msn016>
- Petagna, V. (1787) *Institutiones botanicae*. Napoli, 815 pp.
- Posada, D. & Crandall, K.A. (1998) MODELTEST: testing the model of DNA substitution. *Bioinforma* 14: 817–818.
<http://dx.doi.org/10.1093/bioinformatics/14.9.817>
- Provan, J., Powell, W. & Hollingsworth, P.M. (2001) Chloroplast microsatellites: new tools for studies in plant ecology and evolution. *Trends in Ecology and Evolution* 16: 142–147.
[http://dx.doi.org/10.1016/s0169-5347\(00\)02097-8](http://dx.doi.org/10.1016/s0169-5347(00)02097-8)
- Rambaut, A. & Drummond, A.J. (2007) *Tracer v1.4*. Available from: <http://beast.bio.ed.ac.uk/Tracer>
- Rauwolf, L. (1582) *Augentliche beschreibung der Raiss inn die Morgenlanderin*. Franckfurt Rabe, Franckfurt, 487pp.
- Renner, L. (2004) Plant dispersal across the tropical Atlantic by wind and sea currents. *International Journal of Plant Sciences* 165: 23–33.
<http://dx.doi.org/10.1086/383334>
- Reynolds, J., Weir, B.S. & Cockerham, C.C. (1983) Estimation for the coancestry coefficient: basis for a short-term genetic distance. *Genetics* 105: 767–779.
- Ricciardi, M. (1998) Flora di Capri (Golfo di Napoli). *Annali di Botanica* 54: 7–169.
<http://dx.doi.org/10.1080/00837792.2004.10670763>
- Ricciardi, M., Nazzaro, R., Caputo, G., De Natale, A. & Vallariello, G. (2004) La flora dell'isola d'Ischia (Golfo di Napoli). *Webbia* 59:1: 113.
<http://dx.doi.org/10.1080/00837792.2004.10670763>
- Roth, A.W. (1797) *Catalecta botanica quibus plantae novae et minus cognitae describuntur atque illustrantur*. Vol 1. I.G. Müller, Lipsiae, 244 pp.
- Silvestre, S. (2012) In: Flora iberica *Plantas vasculares de la Península Ibérica e Islas Baleares*. Castroviejo S, Valdes Bermejo E. (eds.), Real Jardín Botánico-C.S.I.C., Madrid 11: 284–286.
<http://dx.doi.org/10.1002/fedr.19870981109>
- Slatkin, M. (1995) A measure of population subdivision based on microsatellite allele frequencies. *Genetics* 139:457–462.
- Smouse, P.E., & Long, J.C. (1992) Matrix correlation analysis in Anthropology and Genetics. *American Journal of Physical Anthropology* 35: 187–213.
<http://dx.doi.org/10.1002/ajpa.1330350608>
- Stefanovic, S.A., Krueger, L. & Olmstead, R.G. (2002) Monophyly of the Convolvulaceae and circumscription of their major lineages based on DNA sequences of multiple chloroplast loci. *American Journal of Botany* 89: 1510–1522.
<http://dx.doi.org/10.3732/ajb.89.9.1510>
- Thompson, J.D., Higgins, D.G. & Gibson, T.J. (1994) *CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice*. *Nucleic Acids Symposium Series* 22: 4673–4680.
<http://dx.doi.org/10.1093/nar/22.22.4673>
- Thorne, J.L., Kischino, H. & Painter, I.S. (1998) Estimating the rate of evolution of the rate of molecular evolution. *Molecular Biology and Evolution* 15: 1647–1657.
<http://dx.doi.org/10.1093/oxfordjournals.molbev.a025892>
- Thorne, J.L. & Kischino, H. (2002) Divergence time estimation and rate evolution with multi locus data sets. *Systematic Biology* 51: 689–702.
- Tiffney, B.H. (1985) The Eocene North Atlantic land bridge: its importance in Tertiary and modern phytogeography of the Northern Hemisphere. *Journal of the Arnold Arboretum* 66:243–273.
- Turrise, R.E. (2001) *Ipomoea imperati* (Vahl) Griseb. (Convolvulaceae), nouvelle entité pour la flore sicilienne. *Flora Mediterranea* 11: 373–378.
- Turrise, R.E. (2007) Importanza della conservazione in situ delle popolazioni di *Ipomoea imperati* (Vahl) Grisebach (Convolvulaceae) della Sicilia. *Bollettino dell'Accademia Gioenia di Scienze Naturali* 40: 141–148.
- USDA (2012) ARS, National Genetic Resources Program. *Germplasm Resources Information Network - (GRIN) [Online Database]*. National Germplasm Resources Laboratory, Beltsville, Maryland. Available from: URL:<http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?406425> (Accessed 15 October 2012).
- Vahl, M. (1790) *Symbolae botanicae*. Copenhagen, 470 pp.
- Wandeler, P., Hoeck, P.E. & Keller, L.F. (2007) Back to the future: museum specimens in population genetics. *Trends in Ecology and Evolution* 22: 634–642.
<http://dx.doi.org/10.1016/j.tree.2007.08.017>
- Weising, K. & Gardner, R.C. (1999) A set of conserved PCR primers for the analysis of simple sequence repeat

polymorphisms in chloroplast genomes of Dicotyledonous Angiosperms. *Genome* 42: 9–19.

<http://dx.doi.org/10.1139/gen-42-1-9>

Wilson, I.J. & Balding, D.J. (1998) Geneological inference from microsatellite data. *Genetics* 150: 499–510.

Xiang, Q.Y., Manchester, S.R., Thomas, D.T., Zhang, W. & Fan, C. (2005) Phylogeny, biogeography, and molecular dating of cornelian cherries (*Cornus*, Cornaceae): tracking Tertiary plant migration. *Evolution* 59:1685–1700.

<http://dx.doi.org/10.1111/j.0014-3820.2005.tb01818.x>