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2About a tetraploid ivy in Sicily: from autochthonous *Hedera* to 3horticultural-invasive-hybrid package?

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10Running title: Tetraploid *Hedera* in Sicily: native or invasive?

11

12Abstract

13In Sicily, *H. helix* is the unique known native species whereas *H. helix* subsp. *poetarum* is
14putatively naturalized in some forests and *H. canariensis* cultivated in various urban's garden.
15Trichome morphology and genome size of some ivies from various west Mediterranean
16forests were compared to Sicilian ones. Ivies from southern Italy, continental France, Corsica
17and Mallorca belong to typical European diploid stellate trichomes *Hedera helix*. Hexaploid
18ivies from southern Spain have been identified as native *H. iberica*. Contrariwise, Sicilian
19ivies studied are related to western European *H. hibernica* (tetraploids with stellate
20trichomes). Is *H. helix* the most widespread and indigenous ivy in Sicily? Therefore, it would
21be the first time that tetraploid would be reported in Sicily where it could possibly correspond
22to an unnoticed autochthonous taxon. However, our results let us think it rather represents an
23invasive which impact on this island rich in endemic species could be considerable.

24

25 **Keywords:** *Hedera*, genome size, trichome morphology, invasive, Sicily

26

27 1. Introduction

28Species belonging to Eurasian genus *Hedera* L. (Araliaceae) are notoriously difficult to
29identify due to high leaves polymorphism caused by environmental conditions and
30heteroblasty: ontogenic change from juvenile to adult leaves (Robbins 1957, 1960; Frydman
31et al. 1973a, 1973b). As heteroblastic development (partially under influence of the hormonal
32production from roots and buds) is reversible (transition from adult to juvenile), we may find
33a great variety of leaves shape within one individual and all along one stem and its branching.

34

35

36According to authors, *Hedera* Eurasian genus would include 12 to 16 taxa (Lum and Maze
371989; McAllister and Rutherford 1990; Vargas et al. 1999; Ackerfield 2001). Thanks to
38trichome morphology - stellate versus scale-like- combined with ploidy levels, recent works
39allow us to better characterize them (Table 1).

40

41The five easternmost species of the genus area distribution present scale like trichomes:
42*Hedera cypria* McAllister (Cyprus), *H. colchica* (K. Koch) K. Koch (South and Est Black Sea
43territories), *H. pastuchovii* Woronow (West and South Caspian Sea territories), *H. nepalensis*
44K. Koch. (Afghanistan to China), *H. rhombea* (Miq.) Bean. (Korean peninsula, Japan,
45Taiwan) (Figure 1a).

46*Hedera helix* L., a diploid stellate trichome plant is present in all European countries: within
47numerous infraspecific taxa described, the most noticed are *H. helix* subsp. *rhizomatifera*
48McAllister in southern Spain and *H. helix* subsp. *caucasigena* Kleop. in Turkey and Caucasus.
49Native *H. helix* s.l. distribution remains unclear in particular where several species and
50infraspecific taxa live in sympatry. In Ireland and in Western part of Great Britain, France and
51Iberian Peninsula grows *H. hibernica* (G. Kirchner) Bean. *H. helix* s.l. and *H. hibernica* are
52invasive species in North America, Argentine, Chile, Australia, New Zealand...

53

54South westernmost part of *Hedera* distribution presents the highest ivies diversity. Each one
55among the three Atlantic archipelagos possess endemic species: *H. canariensis* Willd., *H.*
56*maderensis* K. Koch ex A. Rutherf, *H. azorica* Carr. In North Africa, the genus is represented
57by two endemics species with scale-like trichomes (*H. maroccana* McAllister, *H. algeriensis*
58Hibberd). Five taxa are known in Iberian Peninsula: three stellate trichomes species (*H. helix*
59L. subsp. *helix*, *H. helix* subsp. *rhizomatifera*, *H. hibernica*) and two scale-like trichomes taxa
60(*H. iberica* (Mc Allister) Ackerfield & J. Wen [= *H. maderensis* K. Koch ex A. Rutherf. subsp.
61*iberica* Mc Allister], and locally naturalized *H. maroccana*).

62

63In the field, it is not so easy to identify ivies! Leaves shape varies greatly according to
64environmental conditions (light, humidity), it is different on horizontally and vertically
65crawling shoot -youth leaves much more cut than adult ones. Finally, to observe trichomes, it
66is necessary to use a more powerful magnifying glass than the ones usually available on the
67ground. Trichomes are abundant in apex, more dispersed on young leaves and usually absent

68on mature leaves. Moreover, trichomes fall easily when touched (harvesting, herbarium
69handling...).

70

71In addition, in Europe ivies are largely cultivated for a long time and offered for sale in
72nursery since the 18th century: *H. hibernica*, *H. maroccana*, *H. algeriensis* and *H. colchica*
73have now commonly escaped from garden.

74Furthermore, numerous hybrid and cultivar ivies are introduced, sometimes escape from
75gardens and remain even more difficult to identify. Triploid clones (most of them sterile)
76apparently do not exist in native European population but they are sold in the markets and
77escape in the field. Triploids and particularly allotetraploids may appear spontaneously in
78areas where introduced populations come into contact with native ivies or other previously
79naturalized ivies cultivars. Hybrids individuals appear to be more and more common in
80Europe (Marshall et al. 2017).

81Despite the huge diversity of phenotype and ploidy levels, botanists, naturalists or ecologists
82name generally the ivies indistinctly under the binomial *Hedera helix* L. In this study, we use
83cytometric analyses and trichomes morphology to 1) better identify ivies collected from
84western Mediterranean islands, 2) point out taxonomic inconsistencies and 3) highlight the
85importance of accurate species identification in a context of global change.

86

87 **2. Material and methods**

88 **2.1. Plants studied**

89The origins and numbers of the plants for each of the studied population are shown in Table 2.
90Cytometric analyses were performed on one Iberian population of *Hedera iberica* (Figure 2)
91and various *Hedera* cf. *helix* look like plants from three continental populations (Italy, France)
92and three insular origins: Mallorca, Corsica and Sicily (Figure 1b).

93 **2.2. Genome size analysis by flow cytometry**

94The analyzed leaves were collected in the wild and immediately sent to the laboratory. The
95total nuclear DNA amount was assessed by flow cytometry (Marie and Brown 1993;
96Fridlender et al. 2002). For Iberian ivies, we used *Artemisia arborescens* L. from Crete (2C =
9711.43 pg) as an internal standard, *Petunia hybrida* cv PxPc6 (2C = 2.85 pg) for Sicilian ivies
98and for all other plants *Lycopersicon esculentum* (2C = 2.01 pg). Leaves of the internal
99standard and *Hedera* were chopped using a razor blade in a plastic Petri dish with 600 µl of
100Galbraith nucleus-isolation buffer (Galbraith et al. 1983) containing 0.1% (w/v) Triton X–

101100, supplemented with 10 mM sodium metabisulphite, 1% (w/v) polyvinylpyrrolidone
10210,000 and RNase (2.5 U/ml). The suspension was filtered through 50 µm nylon mesh. The
103nuclei were stained with 75 µg/ml propidium iodide, a specific DNA fluorochrome
104intercalating dye, and kept 15 min at 4°C. DNA content of 5,000–10,000 stained nuclei was
105determined for each sample using a cytometer (CyFlow SL3, Partec, Munster, Germany). The
106total 2C DNA value was calculated using the linear relationship between the fluorescent
107signals from stained nuclei of the *Hedera* individuals and the internal standard. The mean
108value was calculated from measurements of samples comprising 1 to various individuals
109according to populations (Table 2).

110 2.3. Statistical analysis

111The relationship between ploidy P and 2C DNA content x for known ploidy level was
112modeled considering the following quadratic model: $P_i = a + b x_i + c x_i^2 + E_i$. E_i terms are
113supposed to be identically and independently distributed according to a centered Gaussian
114distribution whose standard deviation is indicated by σ . Significance of parameters was tested
115using classical t-test. Statistical analyses were performed using R (R Core Team, 2019).

116 2.4. Trichomes

117Trichomes from young leaves, apex and shoots from dried specimens were observed in SEM
118according to classical microscopical protocol. Measurements of various parameters (arms
119number, length of fused arms, length of free part of arms) from 25 trichomes from Sicily and
12015 from Spain were done, based on Lume and Maze (1989).

121

122 3. Results

123 3.1. Trichomes morphology

124Andalusian ivies have leaves shape with terminal leaf lobe much longer than the others
125(Figure 2) and scale-like trichomes (Figure 3a-b). Trichomes from young dried leaves from
126Los Barrios ivy population have 8-14[17] arms ($m = 9.2 \pm 1.5$) fused in a 48-66 µm central
127disc diameter ($m = 55.2 \pm 7.8$). Arms free length part varies from 55 to 200 µm ($m = 118.7 \pm$
12840.7) with basal width of 14-27 µm ($m = 20.4 \pm 3.49$). Plants do not present difficulty of
129identification based on trichomes morphology (Ackerfield & Wen 2002) and limbs shape
130(Valcárcel et al. 2002): all the individuals correspond to endemic *H. iberica* (McAllister)
131Ackerfield & J. Wen.

132

133Collected plants from Mallorca, Corsica, France and southern Italy have classical *Hedera*
134*helix* leaves polymorphism and stellate trichomes.

135

136Trichomes from Sicilian plants seem more polymorphic most of them are stellate type but
137some are more or less rotate (scale-type related). Unfortunately, most of them fell and
138remained very scarce in leaves. They have 5-8[10] arms ($m = 7.4 \pm 1.37$); practically footless
139(Figure 3f), central disc (welding area of arms) often reduced (26 μ m!), average disc diameter
140 $m = 56.6 \pm 14.2 \mu$ m. The free parts of the different arms of the same trichome are of variable
141lengths: arm length comprised between 63 and 354 μ m ($m = 191 \pm 81.2$) and base width $m =$
142 $18.7 \pm 5.62 \mu$ m. Some arms are partly fused (Figure3d) or bifid beyond the central disc.

143

144Plants from Sicily present stellate type trichomes but majorities of them are more or less
145adpressed (Figure 3c) and then would not match with *Hedera helix* (Ackerfield 2001).
146Moreover, biometric data match better with the ones of *H. hibernica* than *H. helix*: arms more
147numerous and shorter (Lum & Maze 1989). Then, all these characters do not allow us to
148clearly identify species.

149

150 3.2. Doubts and controversies about genome size

151Ivies genomes size presents a good correlation with chromosomes numbers with estimated
152relationship $P_i = 0.916 + 0.920 x + 0.077 x^2$ (adjusted $R^2 = 0.995$, $P < 2.2 \times 10^{-16}$). The
153relationship estimated from our data is mainly linear with a slightly quadratic component
154(Figure 4). All parameters depart significantly from zero.

155

156Our 2C DNA values (Table 2) match well with the data published up to now (Green et al.
1572013, see also Figure 4) when considering 2x to 6x range of ploidy. Based on our model, the
158x genome size given by Green et al. (2013) cannot be accurately predicted.

159

160The "*Hedera helix*" 2C value (8.18 pg) given by Marie & Brown (1993) come from an
161unnamed garden ivy collected in Gif-sur-Yvette (France, Essone), « going to the lab » (S.
162Brown, personal communication). Then, analyzed leaves belong to one of the many ivies
163cultivars that are grown abundantly in the surrounding of Paris region. Moreover, internal
164standard used (*Petunia hybrida*, 2C = 2.85 pg) is not very suitable to measure precisely such
165large genome. Then this value is not exceptional nor atypical as stated by Obermayer and
166Greilhuber (2000) but corresponds probably to an hexaploid cultivar (ca 9 pg). Flow

cytometry is very suitable to estimate ploidy level in *Hedera* genus as triploid of recent hybridization between *H. helix* and *H. hibernica* have a 2C DNA = ca 4.5 pg (Figure 4). On the other hand, *H. colchica* $2n = 8x = 10.3-10.8$ pg (Zonneveld et al. 2005; Green et al. 2013) is lightly different from this pattern, while we would expect a 2C genome size of about 12pg unless plant samples analyzed could be heptaploid hybrids.

172

173 4. Discussion

174

Based on trichomes morphology ivies from France, Italy, Corsica and Balears are *Hedera helix*, DNA amount confirms that they are diploid. Likewise, leaves of Andalusian ivy have an elongated central leaflet, scale-trichomes and plants are hexaploid: they correspond without ambiguity to *H. iberica*.

179

The dimensions of trichomes vary from an author to another (Lum and Maze 1989; Ackerfield 2001; Valcarcel 2002), which is not surprising due to their great variability on the same branch. Besides in *H. helix* and *H. hibernica* there are many trichomes with intermediate aspects (Valcarcel et al. 2002).

Trichomes morphology does not allow to unambiguously name the plants collected in Madonie and Alcamo forests as one of the three known Sicilian species. In fact, trichomes morphology match better to those of *H. hibernica*: arms shorter, more numerous and adpressed. On the other hand, they are tetraploids. On this point, Sicilian ivy do not correspond to any known Sicilian ivies putatively all diploids: native *H. helix* subsp. *helix*, locally naturalized *Hedera helix* subsp. *poetarum* Nyman or cultivated *Hedera canariensis* (Giardina 2007).

191

Sicilian ivies leaves look like to *H. helix* ones and most of trichomes are stellate however they are slightly adpressed. Then, if we combine these characters, ivies collected in the northwestern forests belong to *H. hibernica* as the other known tetraploid ivy species (*H. algeriensis*) have scale trichomes (Table 1). While diploids and hexaploids are known throughout *Hedera* genus range, the two tetraploids ivies are confined to the European Atlantic area and Northern Algeria (Figure 1b).

198

199 From these observations, it is likely that Sicilian ivies are related to *H. hibernica*. However,
200 we cannot also exclude that it would be a native tetraploid with a great biogeographical
201 interest (cryptic apo endemic).

202 Moreover, it could well be a hybrid or a naturalized *H. hibernica* that would have been
203 unnoticed on the island. Of the two populations analyzed in Sicily, none corresponds to *H.*
204 *helix* yet reported as common over a large part of the island.

205 It seems unlikely that this plant is introduced in Madonie (relatively far from urban centers),
206 but Alcamo forest is largely anthropized. *H. hibernica* has been cultivated for a long time
207 everywhere and has become invasive in various part of northern hemisphere.

208 In the highly probable case of a past introduction, field studies should be accompanied by
209 examination of the herbarium samples in order to establish the age of the naturalization. In
210 addition, it seems essential to identify again all the ivies previously reported in the literature:
211 obviously several mentions of *H. helix* correspond to putatively *H. hibernica* (taxonomic
212 confusions). It will allow establishing the status of each of these two taxa.

213

214

215 Widely selected by horticulturists and gardeners, ivies have been the subject of a significant
216 trade since at least the beginning of the 18th century. Since these plants are cheap and easy to
217 grow, it is not surprising that they have become invasive. Indeed, it is well demonstrated that
218 selling price and availability on the market are important factors in their dissemination in the
219 field (Dehnen-Schmutz et al. 2007; Pemberton et al. 2009). In North American, ivies began to
220 naturalize on the East coast of the continent in the 1870s and about 60 years later in the West
221 coast quickly becoming invasive with significant ecological consequences on forest dynamics
222 (Clarke et al. 2006; Green et al. 2013; Strelau et al. 2018).

223

224 *Hedera* are abundant in various habitats in Europe but difficult to identify, that is why the
225 arrival of allochthonous ivies in natural populations passes unnoticed. In the Mediterranean,
226 the situation appears to be even more complex as native taxa possess all the known levels of
227 ploidy.

228 On the French Mediterranean coast, *H. helix* is often located, since it requires relatively mesic
229 forests. English ivy (- or Irish ivy - commercial name for several horticultural plants
230 principally selected from *H. hibernica*) is one of the commonest ivies on the market, most
231 sold in urban landscape for decorative purposes but also for soil stabilization and ground
232 cover. Unfortunately, generalized urbanization and development of road infrastructures

233 facilitate the spreading of those cultivated ivies. It allows contact between introduced ivies
 234 with the natural stands of *H. helix* so that in certain sectors (near cities and residential areas in
 235 particular) the ivies become difficult to characterize (mixture of native species, cultivars and
 236 hybrids). This is patent for example in Southeastern France (see Marseille Table2), Liguria,
 237 Toscana, Lombardia and Lazio where *H. hibernica* s. l. is well implanted around urban centers.
 238 The islands endure the same treatment: in Sicily, ivy cultivars (scale and stellate groups) are
 239 widely planted in archaeological sites! The recent expansion of *Xylella fastidiosa* in Corsica,
 240 introduced from the United States via horticulturists and intensive agricultural practices (Janse
 241 and Obradovic 2010) highlights the massif horticultural imports plants. In Mallorca, the
 242 seaside resorts lead to a standardized landscaping and subsequent horticultural plants
 243 introduction. If in those two islands *H. helix* remains strongly present in natural areas, in
 244 Sicily the ivies of both sampled forests do not correspond to *H. helix*, but to a tetraploid ivy.
 245
 246 These observations are part of a general process in which invasives colonize primarily the
 247 coastal areas and low altitudes (especially if they are strongly anthropized), prior to penetrate
 248 native habitat. The polyploids (in particular 4x) appearing, in addition, over represented
 249 (Verlaque et al. 2002).

250 In Lebanon, where the only reported native species is *H. helix* (Mouterde 1970); we also
 251 noticed various populations of ivies with stellate adpressed trichomes (scale trichomes ivies
 252 are largely cultivated as ornamental from lowland up to 1200m). Then it would be necessary
 253 to clarify if it corresponds to a particular Mediterranean taxon or if it also belongs to this mix
 254 horticultural-invasive-hybrid package.

255

256 Replacement of a mesic native species (like *H. helix*) by a cultivar-invasive-hybrid
 257 consortium that may develop in drier ecosystems may have some ecological consequences.
 258 Rarefaction of wet habitat taxa to the detriment of xerophytics plants is part of a generalized
 259 Mediterranean ecosystems transformation (even putatively well-preserved ones like “relict”
 260 forest or protected areas). It is all the more devious as it goes unnoticed.

261

262 Therefore, fine ivies systematic study is necessary in order to precisely characterize
 263 autochthonous species and understand mechanisms of formation and acclimation of those new
 264 taxonomic entities in the field. These biological invasions contribute to global changes
 265 (change and probable loss of biodiversity...) of which they are powerful indicators. Indeed, if
 266 well identified, they appear as valuable markers of current changes.

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270 Acknowledgements

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272 We thank Spencer Brown (CNRS - Gif sur Yvette) and Nicolas Brouilly (CNRS - Luminy) for
273 their help in flow cytometry and SEM; Fabrice Paraque and André Gilles for English and
274 manuscript revision.

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Table 1 Ploidy level and trichome morphology of ivies (genus *Hedera* L., Araliaceae).

Ploidy level	Diploid 2n = 48	Tetraploid 2n = 96	Hexaploid 2n = 144	Octoploid 2n = 192
Stellate trichomes	<i>H. azorica</i> <i>H. helix</i> L. subsp. <i>helix</i> <i>H. helix</i> subsp. <i>poetarum</i> <i>H. helix</i> subsp. <i>rhizomatifera</i> <i>H. helix</i> subsp. <i>caucasigena</i>	<i>H. Hibernica</i>		
Scale trichomes	<i>H. maroccana</i> <i>H. rhombea</i> <i>H. nepalensis</i> <i>H. canariensis</i>	<i>H. algeriensis</i>	<i>H. cypria</i> <i>H. pastuchovii</i> <i>H. maderensis</i> <i>H. iberica</i>	<i>H. colchica</i>

Table 2 - Origin of studied populations of *Hedera* and Genome size (2C DNA in picograms $\pm$ standard deviation).

n = number of individuals measured. **Marseille:** plants were collected in the center of Massif des Calanques because on the edge of Calanques, ivies are a taxonomic mixture: most individuals are hybrid between native ivy and introduced cultivars, most of them issued from *H. algeriense* and “English ivy”.

Population, putative species	2C DNA $\pm$ SD, in pg
Mallorca , Massanella mount: ca 1100m, <i>Hedera helix</i> L.	3.29 $\pm$ 0.05 (n = 1)
Corsica , Figarella: in <i>Quercus ilex</i> wood, ca m, <i>Hedera helix</i> L.	2.91 $\pm$ 0.05 (n = 3)
France Aix-en-Provence: Wet wood surrounding of Arc river, ca 150m, <i>Hedera helix</i> L. Géménos: wet and fresh forest by Huveaune River ca. 200m; and Sainte Baume <i>Fagus sylvatica</i> forest, ca. 800m, <i>Hedera helix</i> L. Marseille: Calanques, <i>Hedera helix</i> climbing on limestone rocks, ca 30, 80 and 150m, <i>Hedera helix</i> L. Veyre-Monton: wet oak forest dominated by <i>Fraxinus excelsior</i> , <i>Salix fragilis</i> , 480m, <i>Hedera helix</i> L.	3.12 $\pm$ 0.23 (n = 9) 2.98 $\pm$ 0.13 (n = 3) 3.25 $\pm$ 0.22 (n = 2) 3.10 $\pm$ 0.20 (n = 3) 3.59 (n = 1)
Italy , Potenza : Corleto Forest, ca. 800m, <i>Hedera helix</i> L.	2.95 $\pm$ 0.01 (n = 2)
France, Italy, Mallorca, Corsica , <i>Hedera helix</i> L.	3.08 $\pm$ 0.22 (n = 15)
Sicily , Alcamo: forest above village, ca 350m, <i>Hedera</i> sp. Madonie: forest at Piano Zucchi, ca. 1100m, <i>Hedera</i> sp.	5.83 $\pm$ 0.06 (n = 7) 5.82 $\pm$ 0.08 (n = 4) 5.85 $\pm$ 0.03 (n = 3)
Spain , Los Barrios, <i>Quercus suber</i> forest, ca. 50 m, <i>H. iberica</i>	9.21 $\pm$ 0.17 (n = 5)

Figure 1a

Figure 1b

Figure 1 – General outline of native distribution of ivies.

1a - Distribution of the 13 more commonly accepted species of *Hedera*. **1b** - Distribution

map of *H. iberica* (6x) and the two tetraploid ivies species (dotted): Atlantic western

European *H. hibernica* and endemic north Algerian *H. algeriensis*.

Studied populations: Southern Spain hexaploid population from Los Barrios (black dot);

diploid populations from France, Italy, Corsica and Mallorca (with dots); Sicilian tetraploid

populations of Alcamo and Madonie (star).

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 394 **Figure 2** – *Hedera iberica* leaves shape from los Barrios (Spain) population.
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397 **Figure 4** - relationship between genome size (2C DNA) and ploidy level in the genus
 398 *Hedera*.

399 **Continuous line:** estimated relationship ($R^2=0.995$, $P<2.2 \times 10^{-16}$) between genome size and ploidy level. **Dotted**
 400 **lines:** mean confidence prediction for a probability level equal to 0.95. **Black dots:** 2C DNA values (in
 401 picograms) for each plant analyzed. **Grey dots:** genomes size data from Zonnevald et al. (2005) and Green *et al.*
 402 (2013).

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Figure 3 - SEM of *Hedera* trichomes: a-b: Spain, Los Barrios (*H. maderensis* subsp. *iberica*); c-g

Sicily, Madonie and Alcamo (*H. hibernica*).

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