Genetic Variability of an Unusual Apomictic Triploid Cactus—Haageocereus tenuis Ritter—from the Coast of Central Peru

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Abstract

Haageocereus tenuis is a prostrate cactus restricted to a small area of about 2 km² near the city of Lima, Peru. The species is triploid and propagates mainly through stem fragmentation. In addition, propagation via agamospermy is documented and adventitious embryony is also inferred as a mechanism. Although seedling recruitment has not been observed in nature, we have shown that asexually produced seeds are viable. About 45 adult individuals, plus 9 individuals obtained from seeds, were sampled and 5 microsatellite markers were used to assess genetic variability. Microsatellite analysis confirms that individuals from the only existing population are genetically identical and that the population likely represents a single clone. The absence of mutations in any individual, even in highly variable microsatellite loci, may indicate that the species is also of recent origin. Other prostrate species of Haageocereus are suspected to be occasional apomicts. This phenomenon has significant implications for the evolutionary biology and ecology of Haageocereus and other clonal Cactaceae.

Key Words: Cactaceae, clonal propagation, microsatellites, Peru, polyploidy

Haageocereus tenuis Ritter is a prostrate creeping cactus restricted to an area of about 2 km² in a sandy flat close to the city of Lima, Peru (Ostolaza 1990; Ostolaza and Rauh 1990) (Figure 1). This species was found by F. Ritter in 1956 but only described in 1981 (Ritter 1981). Ritter’s description, lacking characters of both flowers and fruits, was completed by Ostolaza and Rauh (1990). The prostrate stems are gray to bluish green, partially covered by wind-blown sand and pieces of shells (Figures 2A,B). They contain up to 15 ribs and are densely covered by grayish spines with microscopic hairs (Figure 2C) presumably functioning in the capture of humidity from fog (Ostolaza and Rauh 1990). Haageocereus tenuis flowers only rarely and never profusely. The flowers are nocturnal, white, funnelform, and up to about 10 cm long. Although they are presumed to be pollinated by bats or moths, no observations have been reported on the pollination biology and reproduction of H. tenuis (Ostolaza 1990).

Clonal propagation was suspected in H. tenuis due to the prostrate habit and sporadic production of flowers and fruits. As in other cacti that reproduce vegetatively (Opuntia spp., Astrocylindropuntia pachyphala, Stenocereus eruca, Lophoicereus schotti) (Grant and Grant 1971, 1980; Parker and Hamrick 1992; Anderson 2001; Clark-Tapia et al. 2005), H. tenuis develops adventitious roots on stems that are in contact with the ground. These stem segments with roots, later detach from the mother plant (Gibson and Nobel 1986; Anderson 2001). The succulent habit allows the new plants to survive until they become well established (Anderson 2001). As suggested for Stenocereus eruca, the low production of flowers and fruits may be a consequence of absence of favorable conditions and resources in an inhospitable habitat. Flower development and seed establishment may be constrained by the limited availability of water and nutrients (Clark-Tapia and Molina-Freaner 2004), whereas the same environmental conditions and the lack of flowers would limit pollinator visitation. The only known existing population of H. tenuis is found at the lower limit of a “loma” formation, an island of vegetation in the desert supported by winter fogs.

Recent chromosome counts revealed that H. tenuis is triploid (2n = 3x = 33) (Arakaki et al. 2007) (Figure 2D).
Taraxacum, one of the best-known triploid apomicts, apomixis allows the reproduction of plants with unbalanced chromosome numbers, in which meiotic disturbances would cause pronounced sterility (Richards 1970). Apomixis is often associated with hybridity and a perennial habit (Stebbins 1950, 1979; Asker and Jerling 1992). A given genotype that is adaptively superior in a certain ecological niche can expand by vegetative reproduction, whereas the production of asexual seeds can increase the plant's capabilities for dispersal, establishment, and range expansion (Grant and Grant 1980; Asker and Jerling 1992).

Apomixis as defined by Stebbins (1950) and Richards (1997) may include both vegetative reproduction and agamospermy (production of fertile seeds without sexual fusion between gametes). Asker and Jerling (1992) restricted the term “apomixis” to agamospermy, arguing that all kinds of situations may exist where vegetative reproduction (i.e., by propagules, stem fragmentation, rhizomes) is only a complement to sexual reproduction. Two forms of agamospermy are recognized: adventitious embryony, when the embryos arise from somatic cells of the nucellus or integument, and gametophytic agamospermy, in which embryo sacs are produced.

Figure 1. Known distribution and ploidy levels of Haageocereus tenuis and putative parental species: H. pseudomelanostele, H. decumbens, H. multicolorispinus, and H. chalaensis (from Calderón et al. 2007 and Arakaki et al. 2007).
Arakaki et al. • Genetic Variability of Apomictic Triploid Haageocereus tenuis

from unreduced initial cells giving rise to plants of the maternal genotype (Stebbins 1950; Asker and Jerling 1992; Richards 1997). Adventitious embryony typically leads to polyembryony, or the formation of more than one embryo per seed. Although polyembryony is typically associated with apomixis, it can also occur in the absence of it (Naumova 1992; Carneiro et al. 2006; Mendes-Rodrigues and Oliveira 2012). Apomicts have also been classified as facultative and obligate, although the latter is considered improbable in nature (Asker and Jerling 1992; Spillane et al. 2001). Apomixis is not rare in angiosperms, including the Cactaceae (Asker and Jerling 1992; Naumova 1992); however, only adventitious embryony has been documented in the family, in Opuntia (Lakshmanan and Ambegaokar 1984; Asker and Jerling 1992; Negrón-Ortiz 1998; Ozias-Akins 2006).

Molecular markers have been used in other Cactaceae to examine genetic variation within and between populations and reproductive systems. For example, the cactus Stenocereus erucus, known as the “creeping devil,” is restricted to inhospitable sandy flats in the Magdalena region of Baja California.

Figure 2. Haageocereus tenuis: (A) in habitat, stems belonging to what was considered a single individual during sampling, (B) stems covered partially with sand and shell fragments, (C) spines emerging from the areole, covered with fine trichomes, (D) chromosomes (2n = 3x = 33), (E) branch with mature fruit, (F) seedling showing 3 cotyledons instead of the typical 2.
and has been considered by Gibson and Nobel (1986) as the most remarkable case of vegetative reproduction in the Cactaceae. However, isozyme studies on the diversity of this species showed moderate levels of genetic variation (higher than those obtained from other clonal cacti) and suggested that both sexual and clonal propagation are present (Clark-Tapia et al. 2005). In spite of this, not all variability can be attributed to sexual reproduction. Various types of somatic mutations also occur in apomictic plants, but the importance of such variation is unknown (Asker and Jerling 1992). In analyses of genotypic variation in 21 clonal plant species (Ellstrand and Roose 1987), all but two revealed multiple clones within and among populations. The two unicolonial species mentioned (Gaura triaingulata and Taraxacum officinum) showed very restricted ecological ranges compared with their congeners.

The only existing population of H. tenuis has declined in about 50% in the last 24 years (Ostolaza C, personal communication), and this trend continues due to pressure by urban development. In the most recent assessment, the conservation status of Haageocereus species by Calderón et al. (2007), H. tenuis has been categorized as critically endangered.

The examination of genetic diversity in H. tenuis using microsatellite markers has the following objectives: 1) to examine genetic variation within the single known population, 2) to assess whether agamospermy occurs, and 3) to discuss the taxonomic and conservation status of H. tenuis.

Materials and Methods

Study Site and Sampling

The site (Figure 1), located in the Province of Huaral (Lima, Peru), close to the town of Chancay, supports the only known population, with about 300 individuals, in an area of approximately 2 km². Plants are partially covered by sand and are difficult to see from long distances. Plants are branching at the base, with branches about 40 cm to 1 m in length. Old branches are brown, woody, and spineless at the base, with new growths, which are green to bluish green and densely covered with gray to whitish soft spines at the tips (Figure 2B). Spines trap sand, small pieces of shells, and feathers/litter carried by the wind from adjacent poultry farms.

Small portions of young stems (approximately 20 cm long) were taken from 45 individuals covering the entire population and growing at least 3 m apart from one another. Stems were dried in silica gel and used for DNA isolation and subsequent microsatellite analysis. Fruits from a plant collected in the same population and maintained in a garden (by Ostolaza C, Figure 2E) were used to obtain seeds and assess the occurrence of agamospermy.

Examination of Apomixis

Mature seeds were dissected to observe polyembryony, a phenomenon frequently associated with apomixis (Asker and Jerling 1992). To examine agamospermy, seeds were germinated and seedlings used to extract DNA and run microsatellites. Root tips were collected and used to count chromosome numbers following Arakaki et al. (2007).

Microsatellite Amplification and Analysis

The general methods employed for microsatellite capture are described in Arakaki et al. (2010). Genomic DNA was extracted using the DNeasy Mini Kit (Qiagen Inc., Valencia, CA). Polymerase chain reaction (PCR) amplifications were performed with 10 µL total volume containing 0.2 units of NEB Tag polymerase (New England Biolabs Inc., Ipswich, MA), 1.5 mM MgCl₂, 0.15 µM of the reverse primer and labeled M13 primer, 0.01 µM of the extended forward primer, and 0.1 mM of each dNTP. PCRs started with a 5-min denaturation time at 94 °C, followed by 40 cycles of 15 s at 94 °C and 3 min at 53 °C, and a final extension step of 5 min at 72 °C. PCR products were run on a CEQ 8000 (Beckman-Coulter, Fullerton, CA) capillary sequencer. We employed microsatellite markers to determine the individuals’ genotypes. These markers were designed for H. tenuis and previously applied in the determination of genetic variation in 3 other species of Haageocereus (Arakaki et al. 2010). Five loci were used for this study (Ht.Id, Ht.Ida, Ht.I, Ht.mi7, Ht.mi11).

Results

Chromosome Counts and Seedling Morphology

We obtained counts for 8 seedlings and all showed the same chromosome number of 2n = 3x = 33 (Figure 2D). Seed germination took 3–7 days, and germination success was 60% (18 of 30 seeds). Six out of the 18 seedlings showed 3 cotyledons instead of the expected 2 (Figure 2F).

Genetic Diversity

All 45 individuals analyzed, plus 9 individuals obtained from seeds, exhibited the same heterozygous genotype, with 1–3 alleles per locus. Three alleles were present in all individuals for loci Ht.Id, Ht.Ida, and Ht.mi11, 2 for locus Ht.Id and 1 allele for locus Ht.mi7 (Table 1). Also in Table 1, we include microsatellite data for sympatric H. pseudomelanostele (Arakaki et al. 2010) for comparative purposes.

Discussion

The genetic variability contained in the single clonal genotype of H. tenuis is highly restricted. In related H. pseudomelanostele, a total of 99 alleles were scored for the same set of loci in 3 populations (with an average of 65 alleles per population) (Arakaki et al. 2010), whereas in H. tenuis, only 12 alleles were amplified (Table 1).

The stable propagation of a genotype with an uneven ploidy level is often regarded as evidence of apomixis itself (Hanna and Bashaw 1987; Bonilla and Quarin 1997). Even if mechanisms may exist for the generation of a certain degree
of variability in populations with uneven ploidy levels (Grant 1981), in our sample this possibility is ruled out by the presence of the same highly heterozygous genotype in all the individuals and indirect evidence of nucellar embryony. Nucellar embryony leads to morphological abnormalities associated with polylembryony, which is the only mechanism of apomixis reported for Cactaceae. Polylembryony in the family has been reported in Opuntia dillenii and at least 5 other species of Opuntia including O. ficus-indica (Maheshwari and Chopra 1955, and references therein; Hanna and Bashaw 1987; Johri et al. 1992). In Opuntia, the zygotic embryo is replaced by several embryos of nucellar origin; however, usually only 1 or 2 such embryos reach maturity fusing and forming seedlings with 3 cotyledons. Most species with adventitious embryony are reported to be pseudogamous, requiring functional pollen for seed development (Richards 1997); however formation of the embryo can occur even in the absence of pollination. In cacti, this might be facilitated by the fact that contrary to the typical angiosperm, where the nutritive tissue in the seed is the endosperm (3n), the food tissue in cacti is the perisperm, derived from cells of the mother plant only (nucellus) and hence diploid (Gibson and Nobel 1986). Alternatively, a limited amount of viable pollen produced by the triploid or compatible pollen from a related species may be sufficient to trigger the formation of viable seeds.

Because H. tenuis is probably of hybrid origin, the amplification of a single allele at locus Ht:mii7 may be due to either a shared allele between the parental species or the inability of the primers to amplify sequences of one of the parental origins. The absence of variability across the entire population, even in highly variable microsatellite loci, may indicate that the genotype is of recent origin and mutation has not generated observable molecular variability yet. Further interpretation of the genetic constitution of this species requires the identification of its genome donors. Since the population is isolated and growing far away from other congeners, possible parental species are difficult to determine. Given its triploid nature, one possibility is that it arose through autopolyploidy by fertilization of an unreduced gamete and a reduced gamete (4n × 2n) (Bretagnolle and Thompson 1995). However, ongoing phylogenetic research (Arakaki et al., unpublished), and a highly heterozygous genotype, suggest that it likely arose through hybridization between a tetraploid and a diploid parent belonging to different species. The tetraploids H. chalaensis and H. multicolorispinus can be proposed as possible parental species. Haageocereus tenuis, H. chalaensis, and H. multicolorispinus thrive on sandy plains at sea level and share several morphological characters, including a prostrate habit. However, H. chalaensis and H. multicolorispinus are currently found about 600 and 500 km south of the known population of H. tenuis, respectively (Figure 1). Two possible diploid parental species are H. pseudomelanostele, which is widespread throughout central Peru, and H. decumbens, also with a prostrate habit and growing in close proximity to H. chalaensis and H. multicolorispinus (Figure 1). Haageocereus pseudomelanostele is an extremely variable, medium-size shrub, with nocturnal or diurnal flowers. Although its morphology is quite distinct from that of H. tenuis, its current distribution is close to that of H. tenuis. The overlap in allele sizes found in H. tenuis and H. pseudomelanostele suggests that the latter cannot be ruled out as a putative progenitor. Hybridity coupled with nucleotypic effects due to the increased DNA contents caused by polyploidy may partly explain the phenotypic differences between the two species. Nucleotypic effects have been shown to have strong influence in phenotype, growth rate, phenology, life cycle, and other aspects of plant development (Bennett 1987). Even when the coast of Peru has been extensively explored, there is always the possibility that there are populations of the putative parental species closer to the location of H. tenuis that we do not know about. Further research is needed, as no conclusive evidence for either an autopolyploid or allopolyploid origin for H. tenuis is available at present.

The production of asexual seeds in a geographically restricted species becomes advantageous by increasing the plant's capabilities for dispersion beyond that accomplished by stem fragmentation. Although 60% of seeds germinated in the laboratory, no seedlings were observed in the field. Recruitment of seedlings has been shown to be low in succulent plants living in arid environments (Mandujano et al. 1996; Turner et al. 1996; Negrón-Ortiz 1998) typically due to ecological stress rather than problems in seed production. In spite of the lack of direct evidence, windows of opportunity may appear for the establishment of new seedlings of H. tenuis in its native location (i.e., years with “El Niño” events, characterized by rains and formation of ephemeral vegetation in otherwise arid areas).

**Implications for Conservation**

Although it is represented by only a single uniclonal population, H. tenuis is morphologically and genetically distinct.

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**Table 1** Multilocus microsatellite genotype of *H. tenuis* and variability found in three populations of a related widespread sexual species (*H. pseudomelanostele*) using the same set of primers

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele 1*</th>
<th>Allele 2*</th>
<th>Allele 3*</th>
<th>Number of alleles and allele size range*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ht.Id</td>
<td>231</td>
<td>257</td>
<td>0</td>
<td>16 (221–253)</td>
</tr>
<tr>
<td>Ht.Ide</td>
<td>199</td>
<td>211</td>
<td>221</td>
<td>13 (197–219)</td>
</tr>
<tr>
<td>Ht.I1a</td>
<td>207</td>
<td>209</td>
<td>211</td>
<td>12 (181–225)</td>
</tr>
<tr>
<td>Ht.mii7</td>
<td>195</td>
<td>0</td>
<td>0</td>
<td>16 (179–203)</td>
</tr>
<tr>
<td>Ht.mii11</td>
<td>237</td>
<td>243</td>
<td>255</td>
<td>42 (227–365)</td>
</tr>
</tbody>
</table>

*Allele sizes in bp.
from other congeners and should perhaps be considered a microspecies. Microspecies, as defined by Grant and Grant (1971) and Grant (1981), are plant populations that reproduce mainly but not exclusively by uniparental methods, are morphologically homogeneous, occupy a restricted geographical area, and are differentiated from related species. Microspecies often have a hybrid constitution that leads to sexual sterility. Other prostrate species of Haageocereus are also suspected to be occasional apomicts. This phenomenon may have significant implications for the evolutionary biology and ecology of Haageocereus and other clonal Cactaceae.

Even in the absence of sexual recombination, clonal species still have a potential to accumulate genetic variation. In a narrowly defined monoclonal microspecies such as H. tenuis, the amount of observable variability is dependent on the resolution of the technique used to assess variability and the period of time since the establishment of the original hybrid (Ellstrand and Roose 1987; Loxdale and Lushai 2003). Long-lived triploids, although highly sterile, may play an important role in the evolution of polyploid complexes by occasionally acting as bridges to higher ploidy cytotypes (Ramsey and Schemske 1998). The evolutionary potential of H. tenuis depends on its long-term survival in nature; however, the preservation of a single known population is currently threatened by urban development.

Calderón et al. (2007) recommended to include the site in the “Reserva Nacional de Lachay,” one of the few protected lomas ecosystems, located 13 km from the H. tenuis population. All species of Haageocereus are considered vulnerable, endangered, or critically endangered, which has motivated ex-situ conservation efforts by botanical institutions to ensure their preservation and future reintroductions (Calderón et al. 2007; Ostolaza C, personal communication). Other alternatives are the relocation of individuals to adjacent areas that are less affected by current threats, and the establishment of simple systems for the capture of fogs to help sustain the population and allow a faster recovery.

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**References**


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