A conservation genetic study of *Rafflesia speciosa* (Rafflesiaeaceae): patterns of genetic diversity and differentiation within and between islands

P.B. Pelser¹, D.L. Nickrent², J.F. Barcelona¹

**Key words**
conservation microsatellites
Negros Panay Philippines population genetics

---

Abstract. *Rafflesia speciosa* is a threatened endo-holoparasitic species. It has several populations in the Central Panay Mountain Range (CPMR) of Panay island and a single population on Negros Island. Because *R. speciosa* is the only Philippine species of the genus that is not endemic to an individual island, it is a suitable species for improving our understanding of the factors underlying the high island endemism of Philippine *Rafflesia*. For this purpose and to inform the conservation management of *R. speciosa*, patterns of genetic diversity and differentiation were studied using 15 microsatellite loci and samples from nine populations. None of these populations shows evidence of inbreeding and *R. speciosa* has similar levels of heterozygosity as generally observed in outcrossing or perennial plant species. The results of AMOVA and Bayesian cluster analyses indicate that the Negros population is genetically differentiated from the CPMR populations. In addition, it has lower genetic diversity than similar-sized *R. speciosa* populations. These findings suggest that sea straits potentially provide significant reproductive barriers to *Rafflesia* species, and are perhaps responsible for their high island endemism. The general lack of genetic differentiation among the CPMR populations as suggested by the AMOVA, PCoA, and STRUCTURE results indicates recent gene flow among them and this finding improves our understanding of the geographical scale and context at which gene flow between *Rafflesia* populations occurs. Conservation efforts should be targeted towards avoiding further habitat degradation in the Negros population. We also recommend protective status for the entire CPMR and reforestation efforts to mitigate the severe habitat fragmentation, destruction, and degradation in this area.

**INTRODUCTION**

*Rafflesia* R.Br. (Rafflesiaeaceae) is a Southeast Asian parasitic plant genus of c. 30 species (Nickrent 1997 onwards). Most of these are only known from individual islands in the Malesian archipelago (Nais 2001, Barcelona et al. 2009b, Bendiksey et al. 2010, David et al. 2011). This pattern of island endemism is perhaps most pronounced in the Philippines, where 12 of the 13 currently recognised species (Pelser et al. 2011 onwards, Galindon et al. 2016, Hidayati & Walck 2016) are only found on a single island (Pelser et al. 2017). Within these islands, several species have very narrow distributions and two are only known from a single population (i.e., *R. auranti*a Barcelona et al., *R. manillana* Teschem., Barcelona et al. 2009a, b, Pelser et al. 2017). Other Philippine *Rafflesia* species are more widespread within an island, but have relatively few and isolated populations. For example, eight species are only known from two or three populations (i.e., *R. baletei* Barcelona et al., *R. consueloeae* Galindon et al., *R. leonardi* Barcelona & Pelser, *R. mixta* Barcelona et al., *R. verrucosa* Balete et al., and *R. schadenbergiana* Göpp. ex Hieron.; Balete et al. 2010, Barcelona et al. 2006, 2008, 2009b, 2011, 2014, Galindon et al. 2016). The rarity of *Rafflesia* species and their disjunct distribution makes them particularly vulnerable to extinction as a result of the on-going destruction and degradation of their habitat (Nais 2001, Barcelona et al. 2009b). The conversion of rainforests for agriculture, mining, and other uses has undoubtedly resulted in local extinction, a reduction of the size of remaining *Rafflesia* populations, and lower genetic connectivity among them (Pelser et al. 2017). The obligate endo-holoparasitic life style of these plants and their pronounced host specificity are factors that further increase extinction risk, because they cannot live in the absence of the few *Tetrastigma* (Mil.) Planch. (Vitaceae) species that are within their host range (Pelser et al. 2016).

Information about the host specificity, habitat preferences, and reproductive biology of *Rafflesia* species can assist their conservation management by revealing the environmental conditions that the populations need to remain viable, thereby providing a better understanding of the factors that explain their current distribution patterns. The results of a recent study of host specificity and host preference of Philippine *Rafflesia* species (Pelser et al. 2016) indicate that all six known host species have widespread distributions within the Philippines. *Tetrastigma* host plants are also more common and widespread than their parasites in other parts of the distribution area of *Rafflesia* (Nais 2001). This suggests that host ranges are not a limiting factor in the distribution of *Rafflesia*, although local differences in host presence and abundance might explain its distribution patterns at the population level (Pelser et al. 2016).

Although detailed research into the habitat requirements of individual *Rafflesia* species has, to our knowledge, not been carried out, it appears that they are not very habitat specific. Members of the genus are confined to tropical rainforest ecosystems, but generally grow in primary as well as secondary forests, have wide elevational ranges, and their hosts are found on various substrates (Nais 2001, Barcelona et al. 2009b, 2011).
preference is therefore an unlikely explanation for the narrow and disjunct distribution patterns of Rafflesia species and their high island endemism.

Instead, it is perhaps more likely that these distribution patterns are a result of poor seed dispersal beyond relatively small distances (Barkman et al. 2017) and the very low probability that Rafflesia seeds are deposited in sufficient proximity of a suitable host plant to result in infection. Very little is known about seed dispersal in Rafflesia. The morphology of their seeds suggests the presence of an elaiosome (Kuijt 1969, Pelser et al. 2013), indicating myrmecochory, and Pelser et al. (2013, present study) indeed observed ants transporting seeds away from Rafflesia fruits (Fig. 1). If ants are the primary seed dispersers, dispersal over large distances, particularly between islands, might be very infrequent. However, evidence of mammalian frugivory (Emmons et al. 1991, Bänziger 2004) suggests that ants might not be the exclusive dispersers of Rafflesia seeds and that seeds might be able to be more readily transported over greater distances (Pelser et al. 2017).

Population genetic data can provide useful context for understanding the factors that have shaped the distribution patterns of Rafflesia species, as well as for more directly informing conservation management by identifying populations that are of conservation interest, such as those showing unique genetic variation (i.e., private alleles), evidence of low genetic diversity and inbreeding, or poor genetic connectivity with other populations (Ellstrand & Elam 1993, Frankham 2005). Population genetic analyses of a microsatellite data set previously revealed low genetic connectivity between populations of the Philippine R. lagascae species complex that consists of R. lagascae s.str., Mt Labo R. lagascae, and R. manillana (Pelser et al. 2017). Furthermore, these analyses indicated that, despite their very close morphological similarity, the allopatric R. lagascae s.str. (Luzon Island) and R. manillana (Samar Island) are reproducing almost allopatrically isolated. In addition, a genetically distinct but morphologically indistinct population of R. lagascae was identified from Mt Labo in the Bicol Peninsula of Luzon, which might represent an undescribed cryptic species. These findings suggest limited effective dispersal of pollen and seeds over larger distances and particularly between islands. Limited seed dispersal was also concluded for two Rafflesia species from Peninsular Malaysia and Thailand (R. cantleyi Solms) and Borneo (R. tuan-mudae Becc.) in a separate study (Barkman et al. 2017). The genetic patterns obtained for the R. lagascae species complex suggest that such limitations might subsequently result in allopatric speciation, potentially explaining the high island endemism of Rafflesia. The discovery of strong genetic differentiation between morphologically similar populations of the R. lagascae complex on different islands also raises the question of whether other currently recognised Rafflesia species with populations on different islands need to be more narrowly circumscribed (Pelser et al. 2017). Rafflesia speciosa Barcelona & Fernando (2002) is such a species and therefore a suitable candidate for further exploring patterns of genetic diversity of this genus in a geographical context.

Rafflesia speciosa is the only Philippine species in the genus that is not endemic to a single island within the archipelago. It is found at c. 350–1100 m a.s.l. in the Central Panay Mountain Range (CPMR) of Panay Island and on Mt Kanlaon in northern Negros Island (Map 1). The forests that are home to R. speciosa are rapidly being fragmented and degraded as a result of land conversion for cattle grazing and other farming practices, road construction, and housing (Fig. 2). At higher elevations, recreational housing, agritourism and ecotourism have recently decimated fragile ultramafic and limestone habitats such that
Fig. 2  a–d. *Rafflesia speciosa*. a. Flower; b. flower in habitat; c. flower bud; d. opened fruit showing small orange-brown seeds. — e–h. Threats to the conservation of *R. speciosa* habitat. e. Mammal trap (Mt Kanlaon Natural Park); f. conversion into agricultural land (barangay Aningalan, San Remigio); g. slash-and-burn practices in Valderrama (smoke of fires in the background, burnt hill slope in the foreground) leave small *Rafflesia*-inhabited forest remnants amidst unproductive grassland; h. a landslide following deforestation (Valderrama).
now only small forest fragments remain. These and other activities have also been reported to negatively impact other species with which R. speciosa shares its habitat (e.g., Oliver 2014, Oliver et al. 1991, 1993, Hamann et al. 1999, Femmer et al. 2000, Gaulke 2010, Mould 2012, Sammler et al. 2012), thereby prompting appeals to declare the CPMR a protected area (e.g., Oliver et al. 1991, 1993). While Sibalum Natural Park (Panay) and Mt Kanlaon enjoy legal protection, these areas are under the constant pressure of, among others, illegal firewood collecting, charcoal making, and hunting (Fig. 2). If R. speciosa shows similar patterns of low genetic connectivity as was observed in the R. lagascae complex, this species is potentially at risk of losing genetic diversity through inbreeding and genetic drift, and might be in need of urgent conservation management.

The objective of this study is to provide the first information about the patterns of genetic connectivity and diversity of R. speciosa with the primary aims of 1) improving our understanding of the factors contributing to the narrow distributions and high island endemism of Rafflesia species; and 2) informing the conservation management of R. speciosa. For this, we use population genetic analyses of a microsatellite data set in which specimens from nine populations are included.

**MATERIALS & METHODS**

**Specimen sampling**

Rafflesia speciosa is known from nine areas (Map 1). One of these is at the foothills of Mt Kanlaon in Negros and the other eight are found in the CPMR in Panay. Three of these are located in the municipalities of Igbaras, Leon, and Miag-ao in Iloilo province at the eastern side of the Range. The remaining five areas are in Antique Province at the western side of the divide. Within the wider Sibalum Natural Park area, R. speciosa sites are concentrated in two areas that are separated by the Tipuluan River. We here refer to the area north of the river as the Mt Poras-Aningalan area, which spans the barangays of Cabladan and Imparayan of Sibalom municipality and barangay Aningalan of San Remigio. The area south of the Tipuluan River is part of barangays Bad-as and Bululaco of Sibalum and referred to as the southern Sibalum Natural Park area. Rafflesia speciosa is further known from the municipalities of Barbaiza, Culas, and Vallederra. Anecdotal evidence suggests that R. speciosa is also present in Laau-an and Tibiao (Antique Province), but these reports remain to be confirmed. For the purpose of convenience, because of difficulties in circumscribing the actual limits of populations, we refer to these nine areas as individual R. speciosa populations.

Following the sampling strategy described by Pelser et al. (2017), tissue samples on silica gel were collected from small parts of two to 16 flower buds or flowers from one or more sites from each of the nine populations (Table 2). The number of samples per population is relatively low, because Rafflesia populations are often small and are sometimes even associated with just a single host plant (Pelser et al. 2017). However, because tissue samples were taken from all or nearly all host plants that were identified in these populations, the percentage of individuals sampled in each population is high. Regardless, our estimates of allele frequencies for small populations should be interpreted with care. Voucher specimens (Table 1) are deposited at CANU, PNH, and SIU.

**DNA extraction and microsatellite genotyping**

Using the DNeasy plant mini kit (Qiagen, Germantown, Maryland), total genomic DNA was extracted from tissue of a total of 105 R. speciosa flowers or flower buds. The results of a pilot study in which the amplification of 17 microsatellite markers developed for R. lagascae (Pelser et al. 2017) was tested (results not reported here) showed that 15 of these loci amplified well for R. speciosa (Man78, Man80, Man109, Man111, Man120, Man142, Man144, Man166, Man171, Man273, Man553, Man714, Man866, Man1134, Man1169), but that the loci Man788 and Man1193 frequently failed to yield unambiguous genotyping profiles. These 15 loci (see Pelser et al. 2017)

<table>
<thead>
<tr>
<th>Population</th>
<th>Voucher (herbarium)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culas, Antique Prov.</td>
<td>Barcelona 3801 (CANU, PNH)</td>
</tr>
<tr>
<td>Barbaiza, Antique Prov.</td>
<td>Barcelona 3791 (CANU, PNH)</td>
</tr>
<tr>
<td>Vallederra, Antique Prov.</td>
<td>Barcelona 3769 with Pelser (CANU, PNH)</td>
</tr>
<tr>
<td>Mt Poras-Aningalan, Sibalum &amp; San Remigio, Antique Prov.</td>
<td>Barcelona 3701 with Pelser (SIU)</td>
</tr>
<tr>
<td>Southern Sibalum Natural Park, Sibalum, Antique Prov</td>
<td>Barcelona 3990 with Pelser (PNH)</td>
</tr>
<tr>
<td>Leon, Iloilo Prov.</td>
<td>No voucher</td>
</tr>
<tr>
<td>Igbaras, Iloilo Prov.</td>
<td>Barcelona 4154 with Pelser (PNH)</td>
</tr>
<tr>
<td>Miag-ao, Iloilo Prov.</td>
<td>Barcelona 4133 with Pelser (PNH)</td>
</tr>
<tr>
<td>Mt Kanlaon, Negros Occidental Prov.</td>
<td>Barcelona 4013 with Pelser (PNH)</td>
</tr>
</tbody>
</table>

* CANU = University of Canterbury Herbarium;  
* PNH = Philippine National Herbarium;  
* SIU = Southern Illinois University Herbarium.  

Table 2  Genetic diversity and number of private alleles observed at 15 microsatellite loci for nine populations of Rafflesia speciosa. Number of Rafflesia samples (number of Tetragastrina host plants from which Rafflesia samples were obtained), percentage of polymorphic loci (P), allele richness (Na), number of effective alleles (Ne), number of private alleles (Na(p)), observed heterozygosity (H_e), expected heterozygosity (H_e), unbiased expected heterozygosity (uH_e), and inbreeding coefficient (F_is) are reported. SE = Standard Error. *Population with a significant deviation from Hardy-Weinberg proportions due to heterozygote deficits after B-Y correction (Narum 2006).
RESULTS

The *R. speciosa* flowers or flower buds that were sampled for this study were obtained from 66 *Tetramystigma* host plants from nine populations. A total of 20 of these hosts belong to *T. harmsdii* Planch. (30 %) and 45 to *T. cf. magnum* Merr. (68 %). One host plant could not be identified to species level. Of the 83 *Rafflesia* specimens for which the sex could be determined, 58 (70 %) were staminate and 25 (30 %) were pistillate. Because we sampled buds or flowers from nearly all infected host plants that we encountered in each population and we did not notice substantial differences in the proportion of infected host plants and those that did not show signs of infection, population sample sizes are assumed to be rough estimates of the relative sizes of *R. speciosa* populations (Table 2).

Genotyping analyses using 15 microsatellite loci showed that 18 of the 105 DNA samples had identical genotypes to those of other flowers or buds obtained from the same host plant. These 18 samples could potentially represent the same *Rafflesia* individual and were therefore excluded from the genetic analyses. A total of 85 of the remaining 87 samples were genetically unique. The two samples that were genetically identical to each other were obtained from different but nearby host plants at the same site of the population.

The 15 microsatellite loci yielded 118 alleles. Across all populations, the number of alleles varied between 4 and 13 per locus, with a mean of 7.87. None of the loci showed evidence of producing null alleles. Only the Valderrama population deviated significantly from Hardy-Weinberg proportions owing to a deficiency of heterozygotes (Table 2). The observed mean heterozygosity (*H*<sub>o</sub>) was slightly higher or lower than two estimates of the mean expected heterozygosity (*H*<sub>e</sub>) (0.58, *uH*<sub>e</sub> = 0.63).

The Mt Poras-Aningalan, Southern Sibalom Natural Park, and Valderrama populations have the highest genetic diversity as measured by the percentage of polymorphic loci, allelic richness, number of effective alleles, and heterozygosity (Table 2). The populations in Culasi, and Miag-ao show somewhat less genetic diversity. The lowest levels of genetic diversity were found in Barbaza and Igbaras, but particularly in Leon and Mt Kanlaon. The inbreeding coefficients are low for all populations (*F*<sub>IS</sub> = -0.30 to -0.06; Table 2).

The number of private alleles per population varies from 0 in Barbaza, Culasi, and Leon, to 6 in Mt Kanlaon and Mt Poras-Aningalan. The results of the AMOVA analysis indicate that the overall genetic differentiation within *R. speciosa* is statistically significant (*F*<sub>ST</sub> = 0.11, *p* < 0.001). A total of 4 % of the genetic variation is found among populations and 5 % among individuals. The AMOVA further shows statistically significant pairwise *F*<sub>ST</sub> values (at *p* = 0.05 after the B-Y correction) between Mt Kanlaon and all other populations, except Leon (Table 3). A total of seven of the pairwise *F*<sub>ST</sub> values between populations

| Table 3 | Pairwise *F*<sub>ST</sub> values between populations of *R. speciosa*. *Significant pairwise comparisons after B-Y correction (Narum 2006). |
|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| **Barbaza**         | **Barbaza**         | **Culasi**          | **Leon**            | **Miag-ao**        | **Igbaras**         | **Mt Poras-Aningalan** | **Southern Sibalom** |
| Barbaza             | 0.013               |                     |                     |                     |                     |                      |                      |
| Culasi              | 0.000               | 0.017               |                     |                     |                     |                      |                      |
| Leon                | 0.000               | 0.042               | 0.006               |                     |                     |                      |                      |
| Miag-ao             | 0.001               | 0.032*              | 0.001               | 0.000               |                     |                      |                      |
| Igbaras             | 0.037*              | 0.039               | 0.015               | 0.000               | 0.036*              |                      |                      |
| Mt Poras-Aningalan  | 0.009               | 0.017               | 0.011               | 0.045               | 0.015               | 0.060*               |                      |
| Southern Sibalom Natural Park | 0.032 | 0.045               | 0.034               | 0.010               | 0.050*              | 0.046*               | 0.052*               |
| Mt Kanlaon          | 0.105*              | 0.114*              | 0.094*              | 0.076               | 0.084*              | 0.086*               | 0.097*               | 0.108* |
in Panay are significant as well. Analyses of the STRUCTURE data for \( K = 1–16 \) in STRUCTURE HARVESTER indicate the presence of four primary genetic clusters (\( K = 4 \); Fig. 3). One of these genetic clusters aligns well with the Mt Kanlaon population. The other genetic clusters do not align with individual populations or groups of populations in Panay. Most of these populations display a large diversity of admixture patterns at the level of individual plants (Fig. 3a). The four genetic clusters also do not align with the two *Tetrastigma* species that were identified as hosts for *R. speciosa* (Fig. 3b). The results of a PCoA of pairwise genetic distances show little genetic differentiation among populations (Fig. 4). The results of the Mantel tests do not reveal a statistically significant positive correlation at \( p = 0.05 \) between geographic and genetic distances between individuals.

**DISCUSSION**

Using data from 15 microsatellite loci, this study presents the first information about the patterns of genetic diversity and genetic differentiation of *R. speciosa*. Although this species is the second-most common species of Philippine *Rafflesia* after *R. lagascae* s.str. from Luzon Island in terms of the number of known populations (9 vs 13; Pelser et al. 2017), its total distribution area (CPMR in Panay and Mt Kanlaon in Negros) is relatively small (Map 1) and all populations are exposed to habitat degradation and fragmentation as a result of slash-and-burn agriculture and other destructive practices (Barcelona et al. 2009b; Fig. 2). Many of the World’s tropical forests experience on-going degradation and fragmentation of their habitat (Turner & Corlett 1996, Laurance 1998, Haddad et al. 2015), which can result in populations that are smaller and more isolated from each other. Such populations are more prone to losing
genetic diversity through genetic drift and inbreeding depression, increasing their extinction risk (Elkstand & Elam 1993, Young et al. 1996, Lowe et al. 2005, Chávez-Pesquera et al. 2014). However, the results of our study indicate that habitat degradation and fragmentation have not yet had a measurable detrimental effect on the genetic diversity of *R. speciosa* populations, because we did not observe evidence of inbreeding as measured by the inbreeding coefficient FIs (Table 2). In addition, *R. speciosa* has very similar levels of heterozygosity (Hs = 0.61, Hr = 0.58, uh = 0.63) as the *R. lagascae* complex (Hs = 0.60, Hr = 0.53, uh = 0.64; Pelser et al. 2017). These values are comparable to those reported for outcrossing or perennial plant species in a meta-analysis of genetic diversity in plants (Nyborn 2004). Under the assumption that our sample sizes are approximately proportional to relative population sizes, smaller populations of *R. speciosa*, particularly in Leon, appear to have somewhat lower levels of genetic diversity, as measured by the percentage of polymorphic loci and allelic richness, than larger populations (e.g., Valderrama). However, this pattern is much less pronounced than what was observed for the *R. lagascae* complex (Pelser et al. 2017). The only *R. speciosa* population known from Negros (Mt Kanlaon) deviates from this pattern by being relatively large, but showing among the lowest levels of genetic diversity when compared to populations from which similar numbers of samples were obtained (Table 2).

In addition to having relatively low genetic diversity, the results of our AMOVA (Table 3) and STRUCTURE (Fig. 3a) analyses indicate that the Mt Kanlaon population is genetically differentiated from the Panay populations of *R. speciosa*. The lack of a statistically significant positive correlation between geographic distances and genetic distances of *R. speciosa* individuals suggests that this might not be due to isolation by distance. Very similar patterns of genetic diversity and genetic differentiation were previously identified for *R. manillana* of the *R. lagascae* complex (Pelser et al. 2017). This species resembles the Mt Kanlaon population of *R. speciosa* in constituting a single population on a different island than where its most closely related populations are found. Whereas all other populations of the *R. lagascae* complex are found on Luzon Island, *R. manillana* is only known from a single population on the nearby island of Samar. Like the Mt Kanlaon population, *R. manillana* has relatively low genetic diversity and is genetically differentiated from populations on the other island, although these patterns are more pronounced in the *R. lagascae* complex than in *R. speciosa* (Pelser et al. 2017). These findings are congruent with the hypothesis that the sea straits that separate islands form significant barriers to gene flow for *Rafflesia* species. Although our results indicate that the Mt Kanlaon population of *R. speciosa* is genetically differentiated from populations on Panay, the lack of distinct clustering of Mt Kanlaon individuals in the PCoA plot (Fig. 4) and the admixture patterns of the Mt Kanlaon population (Fig. 3a) suggest that some gene flow between both islands has occurred. This possibly took place in the late Pleistocene, when the islands of Cebu, Guimaras, Masbate, Negros, and Panay were connected during times with lower sea levels (Negros-Panay Pleistocene Aggregate Island Complex; Brown et al. 2013).

We can, however, not exclude the possibility that the genetic differentiation between the Panay and Negros populations of *R. speciosa* is instead, or in part, due to the natural absence of suitable habitat between the CPMR and Mt Kanlaon. *Rafflesia speciosa* populations have only been found between c. 350 and 1100 m a.s.l., but all land between the CPMR and Mt Kanlaon (including the island of Guimaras, which is located in the Guimaras Strait between Panay and Negros; Map 1) is below 200 m and most of it well below 100 m. However, because other *Rafflesia* species have been found as low as 50 m a.s.l., as well as at higher elevations (e.g., *R. lagascae*) and because almost all forest in Panay and Guimaras at lower elevation has been cleared in historic times (Hamann et al. 1999, Ferner et al. 2000, Sammler et al. 2012), it is also possible that the disjunct distribution pattern of *R. speciosa* is more recent and as a result of the anthropogenic destruction of its forest habitat. The latter explanation has been proposed to explain the genetic differentiation between Panay and Negros populations of two hornbill species, *Penelopides panini* and *Aceros waldeni* (Sammler et al. 2012).

Regardless of whether the genetic differentiation between the Panay and Negros populations is a result of a lack of gene flow across the Guimaras Strait or across unfavourable terrestrial habitats, the results of this study and that of Pelser et al. (2017) improve our understanding of the geographical context and scale at which geographic isolation manifests as genetic differentiation between *Rafflesia* populations. Further context for this is provided by the patterns of genetic differentiation among the Panay populations of *R. speciosa*. Although the presence of private alleles in some of these populations (Table 2) suggest that they are genetically differentiated, the lack of population-level genetic differentiation revealed by the STRUCTURE (Fig. 3) and PCoA (Fig. 4) analyses, the low percentage of genetic variation found among populations (4%; AMOVA), and the absence of statistically significant pairwise FST values between most Panay populations (Table 3), suggests that they recently had relatively high genetic connectivity among them. This contrasts with the low genetic connectivity among populations of *R. lagascae* s.str. (Pelser et al. 2017). Considering that all Panay populations are associated with the CPMR, it is possible that the lack of genetic differentiation in Panay indicates a previously more continuous distribution of *R. speciosa* on the island. If gene flow between these populations is still on-going, the contrast between the patterns of genetic differentiation in *R. speciosa* and *R. lagascae* s.str. might also be explained by the generally smaller geographical distances between *R. speciosa* populations than between populations of *R. lagascae* s.str. These distances might be small enough for its seed dispersers to cross. However, since habitat destruction and fragmentation in the CPMR are quite severe (e.g., Oliver et al. 1991, 1993, Hamann et al. 1999, Ferner et al. 2000, Gaulke 2010, Mould 2012, Sammler et al. 2012, Oliver 2014), *R. lagascae* s.str. is less likely to be dispersed by ants (Fig. 1) or mammals, it is perhaps more likely that gene flow across deforested terrain primarily happens by means of pollen dispersal by flies. *Rafflesia* pollen is dispersed by carrion flies (Calliphoridae), which are attracted to the rotting odour of *Rafflesia* flowers and the visual appearance of their large red flowers (Bänziger 1991, Nais 2001). These flies are long-lived and strong fliers, and can travel distances of 22 km in a few days (Bishop & Laake 1921, Bänziger 1991). They have therefore been considered to be able to disperse *Rafflesia* pollen over larger distances (Bänziger 1991, Nais 2001). If the unconfirmed report of *R. speciosa* in Tibiao is correct, all populations in the CPMR are within 22 km of one or more of the other populations, so it is possible that pollen dispersal currently plays a more important role in sustaining gene flow between populations of *R. speciosa* than seed dispersal. This hypothesis, however, remains to be tested.

*Rafflesia speciosa* exclusively parasitizes two *Tetragastrigma* species (T. harmandii and T. cf. magnum), although it is sympatric with four additional species (Pelser et al. 2016). When different populations of a parasite species evolve preferences for different species within their host range, this can result in genetically distinct groups within the parasitic species (i.e., host-race formation; De Vega et al. 2008, Norton & Carpenter 1998, Román et al. 2007, Pelser et al. 2016). These host races
might eventually evolve into different species (Amico & Nickrent 2009). The STRUCTURE results (Fig. 3b), however, fail to show that R. speciosa individuals growing on T. harmandii are genetically differentiated from those infecting T. cf. magnum plants and therefore demonstrate that host-race formation cannot explain the genetic patterns observed for R. speciosa.

In conclusion, our study resulted in a better understanding of the patterns of genetic diversity and differentiation of R. speciosa in the context of its current distribution. When compared to those of the R. lagascae complex (Pelsers et al. 2017), our results indicate that although Rafflesia species are typically rare, have restricted and fragmented distributional ranges, show high island endemism, and have small population sizes, they may still retain similar levels of genetic diversity as outcropping and perennial plant species. In addition, closely related populations on different islands are genetically differentiated from each other. This finding is compatible with the hypothesis that sea straits form significant barriers to gene flow in Rafflesia, although, in the case of R. speciosa, the genetic differentiation between Panay and Negros can also be explained by the current presence of unfavourable habitat between these two islands. Finally, our results contribute to a better understanding of patterns of genetic differentiation among Rafflesia populations in different geographical contexts, with R. speciosa showing stronger genetic connectivity among populations within a single mountain range, and R. lagascae displaying less genetic connectivity between generally more distant populations belonging to different mountains and mountain ranges (Pelsers et al. 2017).

Our results show that particularly the R. speciosa populations in two protected areas (Sibalom Natural Park and Mt Kanlaon Natural Park) and Valderrama contain private alleles (Table 2). These areas are therefore of noted conservation significance as sources of unique genetic diversity. The Mt Kanlaon population, however, has relatively low genetic diversity and this together with its isolated location make it vulnerable to further reductions in genetic diversity as a result of inbreeding and genetic drift. It is located close to settlements and agricultural land and therefore exposed to human activities that result in habitat degradation. This previously included the conversion of forest into plantations of exotic tree species for forestry, but currently perhaps mostly illegal firewood collecting, charcoal making, logging, and hunting (Fig. 2). The most significant threats to the populations in Panay, including those in Sibalom Natural Park, are habitat degradation and fragmentation resulting from land conversion to farms, roads, and settlements, as well as wildfires that spread from burning nearby grasslands (Fig. 2). Cattle are also often allowed to graze inside the remaining patches of native forest and this has resulted in severe habitat degradation. In some areas, grazing of understory plants appears to have negative impacts on natural forest regeneration. Particularly in Valderrama, deforestation on steep slopes prompted landslides that damaged nearby forest patches (Fig. 2). Rafflesia speciosa populations in Panay that enjoy partial legal protection currently still display genetic connectivity and therefore are exposed to human activities that result in habitat degradation and fragmentation resulting from land conversion to farms, roads, and settlements, as well as wildfires that spread from burning nearby grasslands. Cattle are also often allowed to graze inside the remaining patches of native forest and this has resulted in severe habitat degradation. This previously included the conversion of forest into plantations of exotic tree species for forestry, but currently perhaps mostly illegal firewood collecting, charcoal making, logging, and hunting (Fig. 2). The most significant threats to the populations in Panay, including those in Sibalom Natural Park, are habitat degradation and fragmentation resulting from land conversion to farms, roads, and settlements, as well as wildfires that spread from burning nearby grasslands. Cattle are also often allowed to graze inside the remaining patches of native forest and this has resulted in severe habitat degradation. In some areas, grazing of understory plants appears to have negative impacts on natural forest regeneration. Particularly in Valderrama, deforestation on steep slopes prompted landslides that damaged nearby forest patches (Fig. 2). Rafflesia speciosa populations in Panay that enjoy partial legal protection currently still display genetic connectivity and do not show evidence of inbreeding. However, on-going habitat destruction, fragmentation, and degeneration are undoubtedly bringing this species closer to extinction, as is the case with other threatened species that share its natural habitat (e.g., Walden’s Hominb, the Visayan spotted deer, the Panay monitor, and the Visayan warty pig). In our opinion, this downward trend can only be halted if the entire CPMR receives effective legal protection and the loss of genetic connectivity between R. speciosa populations is prevented by creating corridors of native forest between the remaining forest fragments.

**Acknowledgements**

This project was funded by the Marsden Fund Council from Government funding administered by the Royal Society of New Zealand, and by the Linnean Society, Percy Sladen Memorial Fund, and the National Geographic Foundation. We are grateful to the Department of Environment and Natural Resources (DENR) and in particular M.S. Lim, Director of the Biodiversity Management Bureau (BMB) and staff of the DENR regional and local offices for facilitating the issuance of collecting, transport and export permits. Prior Informed Consents (PICs) were issued by the Protected Areas and Management Boards (PAMB) of Sibalom Natural Park and Mt Kanlaon Natural Park, and the Mayors of Barbaça, Culi, Ibarra, Leon, Moaac, San Remigio, Sibalom, and Valderrama. We would also like to thank Noe Gapas of the National Museum, L.J. Paguntalan and P.G.C. Jakosale (Philippine Biodiversity Conservation Foundation) for helping us secure a PIC from Mt Kanlaon Natural Park. R.P.M. Loliña provided advice about locality names in Panay. Numerous field guides/assistants and staff of DENR, especially A. Fernando and A. Rafa, and ULMC deserve our sincerest gratitude, for without their help, these people would not be possible.

**REFERENCES**


