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How sympatric is speciation in the *Howea* palms of Lord Howe Island?

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Abstract

The two species of the palm genus *Howea* (Arecaceae) from Lord Howe Island, a minute volcanic island in the Tasman Sea, are now regarded as one of the most compelling examples of sympatric speciation, although this view is still disputed by some authors. Population genetic and ecological data are necessary to provide a more coherent and comprehensive understanding of this emerging model system. Here, we analyse data on abundance, juvenile recruitment, pollination mode and genetic variation and structure in both species. We find that *Howea forsteriana* is less abundant than *Howea belmoreana*. The genetic data based on amplified fragment length polymorphisms markers indicate similar levels of variation in the two species, despite the estimated census population size of *H. belmoreana* being three times larger than that of *H. forsteriana*. Genetic structure within species is low although some weak isolation by distance is detectable. Gene flow between species appears to be extremely limited and restricted to early-generation hybrids – only three admixed individuals, classified as F2s or first generation backcrosses to a parental species, were found among sampled palms. We conclude that speciation in *Howea* was indeed sympatric, although under certain strict definitions it may be called parapatric.

Keywords: genetic differentiation, *Howea*, Lord Howe Island, pollination, speciation, sympatry

Introduction

Sympatric speciation remains one of the most controversial issues in evolutionary biology (Coyne & Orr 2004). Although its occurrence is not so strongly contested nowadays, our understanding of the frequency and mechanisms of the process is still limited and there are very few unequivocal, well-documented cases (Bolnick & Fitzpatrick 2007). One example, involving two species of the palm genus *Howea* (Arecaceae), was recently reported (Savolainen *et al.* 2006) from Lord Howe Island (LHI), a minute (14.5 km^2) volcanic island in the Tasman Sea 580 km off the east coast of Australia. This case study, the first in plants that does not involve polyploidy, is now regarded by many as one of the most compelling instances of sympatric speciation (Gavrilets & Vose 2007) and has already become a textbook example of the process (Barton *et al.* 2007; Stuessy 2008; D. Futuyma, personal communication). However, this view is also disputed by some authors (e.g. Stuessy 2006).

Howea palms are the best known and most emblematic of LHI's endemic plants. The two species of *Howea*, *Howea belmoreana* (*Hb*) and *Howea forsteriana* (*Hf*), are a prominent feature of the landscape of LHI (Pickard 1983). The *Howea* palms have also long played an important role in the livelihoods of the LHI islanders.

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The first houses on the island were built from *Howea* trunks and thatched with their leaves (hence the common name, thatch palm, for *H. forsteriana*) (Hutton 1998). The seeds and more recently seedlings of *Howea* have been traded since the 19th century (Hutton 1998). *Howea forsteriana*, also known as the kentia palm, was greatly favoured by the Victorians for its elegant form and hardiness and has since become one of the world's most commonly cultivated indoor plants. It is one of the most important and lucrative species in trade. A substantial proportion of world demand for *H. forsteriana* is supplied directly from LHI. To add value, sprouted seedlings rather than seed are marketed from a nursery run by the LHI local government, the sole authorized commercial distributor of palms on the island. Between 2000 and 2005, an average of 3.2 million seeds of *H. forsteriana* was harvested on LHI for seedling production, 95% coming from wild stands (L. Wilson, personal communication). Small amounts of seeds of *H. belmoreana*, which is less popular in trade, are also harvested. Thus, sustainable harvesting, management and protection of *Howea* are not only crucial from a conservation perspective, but are also vital for local and global economies.

Currently, both species are still widespread and are often found growing together throughout LHI, although some soil preferences have been observed (Savolainen *et al.* 2006). *Howea forsteriana* is particularly abundant at lower elevations, especially on calcareous soils on calcarenite where it typically forms dense, pure stands, but it also occurs up to 350 m. *Howea belmoreana* tends to favour basaltic soils up to 400 m where it is usually mixed with other rainforest tree species (Green 1994; Savolainen *et al.* 2006). Using a combination of phylogenetics and ecological field studies, Savolainen *et al.* (2006) demonstrated that the two species are sister taxa, occur sympatrically, are strongly reproductively isolated and that a prior allopatric phase was unlikely, thus fulfilling established criteria for sympatric speciation (Coyne & Orr 2004). In addition, using an amplified fragment length polymorphisms (AFLP) genome scan they demonstrated that only a small proportion of loci are the likely targets of divergent selection (Savolainen *et al.* 2006).

Amplified fragment length polymorphisms data can provide a wealth of information on genome-wide variation, genetic structure and gene flow both within and between species. In addition, few data on the ecology of *Howea* palms have been published so far. Savolainen *et al.* (2006) demonstrated an association of each species with a different soil type and phenological differences, but did not study pollination and demography thoroughly. These genetic and ecological aspects, as yet not addressed by Savolainen *et al.* (2006), need to be studied in *Howea* to provide a coherent and comprehensive understanding of this emerging model system of speciation (Gavrilets & Vose 2007).

In this study, we ask a series of questions about speciation in the *Howea* palms, emphasizing the phylogeography, microhabitat use, pollination biology and abundance, of the two species. The aim of this study was to provide crucial information for the debate as to whether speciation in *Howea* was parapatric (Gavrilets & Vose 2007) or even allopatric (Stuessy 2006), as opposed to sympatric *sensu stricto*. Although Savolainen *et al.* (2006) reported that their field survey identified only a few hybrids, here we ask whether genetic data and tests for gene flow within and between species are supporting this view. We extend the preliminary assessment of population structure of Savolainen *et al.* (2006) to evaluate how much geographic differentiation, if any, is present on LHI. We study the demographic distribution of palms throughout LHI and assess juvenile recruitment in local populations, as a critical indicator of population status and possibly adaptation to soil types. Finally, we report on some experiments to determine the pollination mechanism of *Howea*.

Materials and methods

Population genetic analyses

A total of 94 *H. belmoreana* and 105 *H. forsteriana* individuals were sampled for AFLP analysis as described in Savolainen *et al.* (2006). Figure 1 provides the 24 locations where palms were sampled for DNA analysis, and which encompass the many parts of LHI where *Howea* is present. Here, allele frequencies were computed with the Bayesian estimator of Zhivotovsky (1999) for dominant markers and expected heterozygosity with the unbiased estimator of Lynch & Milligan (1994) as implemented in AFLP-SURV (Vekemans *et al.* 2002). We also report direct-count proportions of polymorphic bands. Because of their dominant character, the inbreeding coefficient cannot be directly computed from AFLP data, and current methods for its estimation are not reliable (Holsinger & Lewis 2007). However, Zhivotovsky's method works well even in the case of moderate departures from Hardy–Weinberg equilibrium. The palms are wind-pollinated and thought to be largely outcrossing, which should limit inbreeding, thus we decided also to use allele frequencies estimated by the Bayesian method to estimate F_{ST} values.

We tested for the presence of isolation by distance in each species using (i) the Mantel test in Arlequin (Excoffier *et al.* 2005) to assess correlation between the matrix of log geographic distances and the $F_{ST}/(1 - F_{ST})$ matrix and (ii) regression of the pairwise kinship

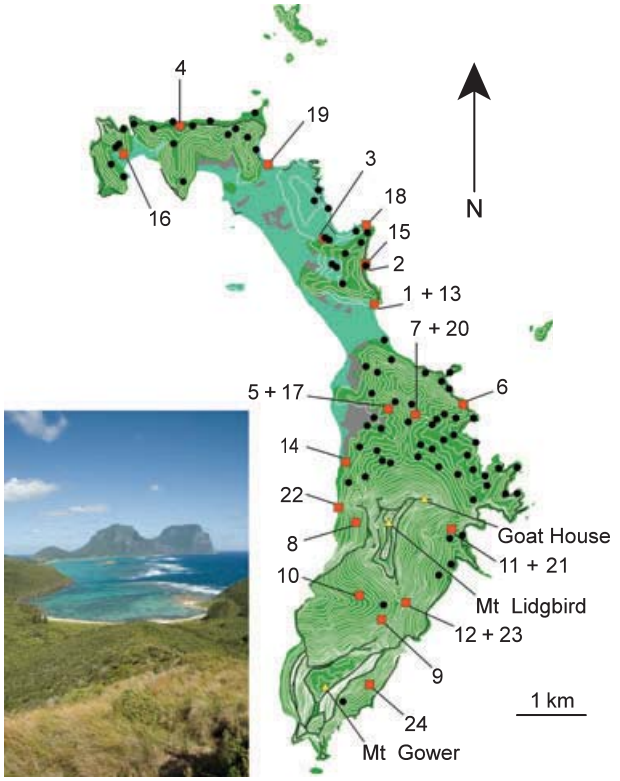

Fig. 1 Map of Lord Howe Island showing the location of: (i) the 24 sites where palms were sampled for DNA work (red squares; no. 1–12 *H. belmoreana*; no. 13–24 *H. forsteriana*; numbering as per Table 3); (ii) the 79 quadrats were population data were recorded (black circles; full details in Supporting Information). Although calcarenite and volcanic soils and intermixed in most of the lower parts of the island where *Howea* grows, shaded green areas indicate volcanic rocks whereas turquoise indicates areas that are predominantly occupied by calcarenite. Note that the settlement is also situated on the turquoise area, hence the restricted number of sampling locations. The inset shows Mount Lidgbird (left) and Mount Gower (right) in the distance (photo W. J. Baker). See text for further details.

measure of Hardy (2003) on log geographic distance (km) in SPAGEDI v1.2 (Hardy & Vekemans 2002) with jackknifing over loci. To determine values of dispersal (i.e. *s*, the root mean square parent-offspring distance; see Vekemans & Hardy 2004), we needed an estimate of the effective palm density, which we evaluated based on counting palms in 79 quadrats of 20 × 20 m (Fig. 1 and see below). Results from analysis of molecular variance (AMOVA) were previously reported by Savolainen *et al.* (2006).

The individual-based Bayesian clustering method implemented in Structure 2.2, which explicitly takes into account the dominant data (Falush *et al.* 2007), was used to test for the presence of genetic clusters. Analyses were run on three data sets: (i) the full data set (*H. belmoreana* + *H. forsteriana*), (ii) *H. belmoreana* alone and (iii) *H. forsteriana* alone. We ran analyses with K = 1–6 clusters in each data set, and additionally models with K equal to the number of sampled sites (N), $N - 1$ and $N - 2$. The admixture model with correlated allele frequencies was used with prior settings as suggested by Falush *et al.* (2007). Each analysis was repeated at least three times and, after preliminary runs, we used 10 000 steps of burn-in and 100 000 subsequent iterations. We applied two approaches to infer the most likely number of genetic clusters in each data set. First, the log probabilities of the data given K[ln $P(X|K)$] were compared for different values of K. Second, ΔK, the second order rate of change of ln $P(X|K)$ was used (Evanno *et al.* 2005). Although ΔK may be a better criterion, it cannot be computed for K = 1 and for the highest number of tested K.

In addition to the Structure analysis we used the program NEWHYBRIDS (Anderson & Thompson 2002) on the entire data set. The method allows one to identify, given enough information in the data, hybrids and backcrossed individuals up to the indicated generation. Importantly, it can distinguish between F1, F2 and backcross individuals (Anderson 2008). We ran this analysis multiple times with uniform priors, 2×10^4 iterations of burn-in were followed by *c.* 2×10^5 subsequent iterations to estimate posterior probabilities. We neither provided *a priori* classification of any individual nor refined priors on allele frequencies. The aim of this study was to identify hybrids of different classes (F1, F2 and backcrosses in both directions).

Population structure and abundance

In addition to the 24 sites in which palms were sampled for AFLP studies, population data were gathered from 79 quadrats (20 × 20 m) sampled throughout the island (Fig. 1 and Supporting Information). Quadrat locations were generated at random from a geographical information system. However, for reasons of logistics and safety, data gathering in the more accessible northern part of the island (north of the Goat House Cave; Fig. 1) were prioritized. Quadrats located in dangerous sites or in areas disturbed by human habitation were excluded. At each site, the number of adults of each *Howea* species was counted as well as the number of juveniles (defined as a palm lacking an aerial stem). Soil pH (a proxy for underlying geology, i.e. acidic soils over volcanic rocks, basic soils over calcareous rocks), altitude, vegetative and other descriptive data were collected, and the precise geo-reference of the location confirmed with a GPS receiver (Supporting Information). These data give us insights into the functioning of the palm populations and their relative abundance.

A general linear model was used to relate the abundance of juveniles to the abundance of adults, soil pH and altitude. Abundance data were square root transformed prior to analysis.

Pollination

To assess the role of wind and animals in pollination, a series of exclusion experiments was conducted. Inflorescences were covered in paper bags to exclude both wind- and animal-borne pollen. Fine mesh bags (mesh size <0.5 mm) were used to exclude animals, but permit wind-borne pollen. Control inflorescences were left unbagged for open pollination. Exclusion experiments for *H. forsteriana* were conducted in a plantation established on LHI for seed production. The plantation was located in a deforested site, near the LHI research station, which was previously occupied by natural vegetation with abundant *H. forsteriana*. Plantation palms flower while still short in stature, unlike wild palms, making inflorescences accessible and the experiment feasible. The inflorescence of *H. forsteriana* comprises several spikes united at the base. The two exclusions and the control were applied to spikes within a single inflorescence. The bags were applied in advance of female flower maturation and were removed 1 week after the end of receptivity, as indicated by the stigmas shrivelling and turning brown. Five weeks later, the number of developing fruit was counted as well as the total number of female flowers (equivalent to number of setting fruit plus the number of female flower scars). It was not possible to apply the same experimental approach to *H. belmoreana* because palms flowering at an accessible height were not available, necessitating tree-climbing and because, unlike *H. forsteriana*, the inflorescences consist of single spikes and are fewer in number. Consequently, a smaller number of inflorescences were treated and it was not feasible to apply both treatments and a control to any individual palm.

Results

Distribution, abundance and juvenile recruitment

Among the 79 quadrats, 67% contain *Howea* palms (Supporting Information). *Howea belmoreana* is more widespread, being present in 52% of the quadrats, while *H. forsteriana* is more restricted, occupying only 29% of the quadrats. The numbers of adults per quadrat (in occupied quadrats) were for *H. belmoreana*: mean 40, SD 30, range 2–104, $n = 41$ and for *H. forsteriana*: mean 24, SD 30, range 1–105, $n = 22$ (Supporting Information). We note that part of the northern half of the island was sparsely sampled because of disturbance of random quadrat localities by human habitation (Fig. 1). This area is dominated by calcarenite geology upon which *H. forsteriana* is often a dominant vegetation component. Thus, the distribution of *H. forsteriana* may be slightly underestimated.

The presence of juveniles is a key measure of regeneration and population status in palms. All quadrats containing adult *H. belmoreana* also contained at least some juveniles (Supporting Information). This suggests that regeneration is good. In contrast, 30% of quadrats with adult *H. forsteriana* palms had no juveniles.

Of the three tested predictors of the juvenile abundance (number of adults of a given species, pH and altitude), only the number of adults was highly significant (Table 1). The relationship between abundance of juveniles and adults was linear (Fig. 2). One outlier quadrat with 51 adults and a surprising number of juveniles (i.e. 612) of *H. forsteriana* is not represented in Fig. 2. However, this outlier demonstrates that juvenile density can be very high. Also, the relationship between the number of adults and the number of juveniles does not plateau for high numbers of adults (Fig. 2). This indicates that juvenile density is not limited by competition or other density dependent factors, but could be limited by seed availability and/or seed/juvenile survival on different soil types.

Pollination

The two exclusion treatments and a control were applied to one inflorescence on each of 24 individual of *H. forsteriana* (Table 2). Differences among treatments were highly significant (Friedman ANOVA $P < 10^{-5}$). Fruit set for animal-exclusion treatments was significantly higher than fruit set of the open pollination (Wilcoxon matched pair test $P < 10^{-4}$). Complete exclusion treatments yielded no fruit in 21 individuals and only 1–2% fruit set in three others, the latter most likely because of contamination with pollen before the bag was applied. Our results for *H. belmoreana* are less complete, but are consistent with those from *H. forsteriana* (Table 2). These results indicate that *Howea* is wind-pollinated

Table 1 General linear model explaining the abundance of juveniles of both species in the quadrats. Numbers of adults and juveniles were square root transformed

	No. adults (of the same species)	pH	Altitude
H. forsteriana	$F_{(1,72)} = 74.44$ $P < 10^{-6}$	$F_{(1,72)} = 1.36$ $P = 0.24$	$F_{(1,72)} = 0.01$ $P = 0.95$
H. belmoreana	$F_{(1,72)} = 301.00$ $P < 10^{-6}$	$F_{(1,72)} = 1.93$ $P = 0.17$	$F_{(1,72)} = 0.12$ $P = 0.73$

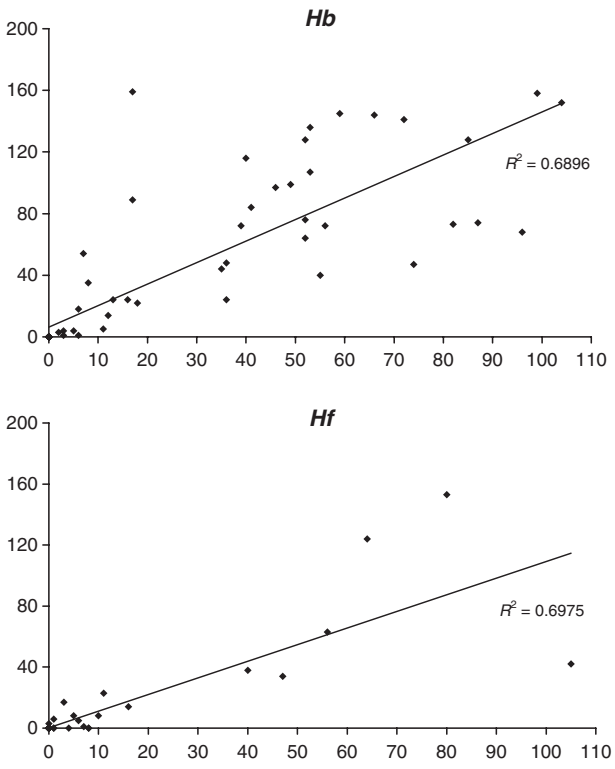

Fig. 2 Relationship between the number of adults and juveniles per quadrat in *Howea* palms (*Hf* = *H. forsteriana*, *Hb* = *H. belmoreana*; R^2 = squared correlation coefficient).

and is not apomictic. The higher fruit set observed in *H. forsteriana* for the animal-exclusion treatments over open pollination may be due to the elimination of flower/fruit predators.

Genetic variation

Levels of variation were similar in the two species: 293 polymorphic loci (173 or 37.5% at 95% criterion) in *H. belmoreana* and 303 (186 or 40.3% at 95% criterion) in *H. forsteriana* from 625 bands scored. Nei's unbiased gene diversity was $H = 0.121$ (SE 0.007) in *H. belmoreana* and 0.135 (0.008) in *H. forsteriana*. Gene diversity at individual sites ranged from 0.105 to 0.165 in *H. belmoreana* and from 0.092 to 0.194 in *H. forsteriana* (Table 3 and Fig. 1).

Population structure, hybridization and isolation by distance

When all sampling sites of each species were pooled and allele frequencies were estimated according to the Bayesian approach, then $F_{ST} = 0.495$ (SE 0.028), indicating strong differentiation between genomes of *H. belmoreana* and *H. forsteriana*. F_{ST} estimates within species were much lower, for *H. belmoreana* $F_{ST} = 0.054$ (0.108) and for *H. forsteriana* = 0.132 (0.000). This is in agreement with the AMOVA reported by Savolainen *et al.* (2006), which detected that only 5.4% of total variation was accounted for by populations within species in contrast to 62.6% accounted for by interspecific differences.

Individual-based Structure analysis of the entire data set when $K = 2$ distinguished two clear-cut groups and received the highest support ($\Delta K = 332.3$). These two clusters corresponded to *H. belmoreana* and *H. forsteriana*. Only three individuals, two *H. belmoreana* and one *H. forsteriana* showed substantial genetic admixture and may represent hybrids (Fig. 3). On the basis of ln $P(X|K)$ criterion, the most likely number of clusters was four (Table 4), and it shows that there is some genetic structure in *H. forsteriana* (Fig. 3). However, ΔK gives overwhelming support to $K = 2$ (Table 4). To further explore the possibility of a subtler intraspecific structuring, we also performed the analysis for each species separately. In *H. belmoreana*, the most likely K was 1 indicating the lack of genetic structuring. For higher K, only two possible hybrids had substantial fraction of their ancestry in separate clusters. ΔK was similar for a number of K-values (Table 4). As already

Table 2 Results of experiments on the pollination mode

Treatment	No. individual treatments	Proportion of fruit set		Average set fruits/inflorescence	
		Mean	SE	Mean	SE
H. forsteriana					
Test for apomixis	24	0.002	0.001	0.2	0.1
Insect exclusion	24	0.531	0.048	143.0	7.9
Control	24	0.332	0.041	133.3	6.3
H. belmoreana					
Test for apomixis	7	0.005	0.005	0.9	0.9
Insect exclusion	6	0.314	0.067	65.8	15.6
Control	4	0.491	0.120	89.3	39.3

Table 3 Genetic variation in species and populations. Percentage of polymorphic loci is given both as a direct count and according to the 95% criterion based on allele frequencies computed using the Bayesian method of Zhivotovsky (1999). Large discrepancies between the two measures in most cases are apparently the result of the estimation procedure, which assigns frequencies slightly >0.05 for the recessive allele at loci fixed for the 'band present' allele within a population. *Hb*, *H. belmoreana*; *Hf*, *H. forsteriana*

Population	*n*	*P*	*P* 95%	*H*	SE(*H*)
Hb	94	63.6	37.5	0.121	0.007
01	8	23.2	51.2	0.123	0.007
02	10	22.0	51.2	0.105	0.006
03	3	14.1	47.7	0.141	0.007
04	9	23.2	52.1	0.115	0.007
05	7	21.7	51.2	0.124	0.007
06	10	26.4	57.0	0.118	0.007
07	11	24.5	55.5	0.112	0.006
08	8	34.3	55.1	0.159	0.008
09	7	20.6	50.3	0.116	0.007
10	6	26.8	48.8	0.165	0.008
11	10	22.5	50.1	0.114	0.007
12	5	17.1	47.5	0.120	0.007
Hf	105	65.7	40.3	0.135	0.008
13	10	24.7	51.2	0.125	0.007
14	9	17.1	17.1	0.092	0.006
15	8	19.5	46.4	0.113	0.007
16	10	20.2	46.9	0.108	0.007
17	10	19.9	49.2	0.110	0.007
18	11	20.9	17.1	0.105	0.007
19	9	25.2	46.6	0.131	0.008
20	10	23.5	51.8	0.116	0.007
21	8	18.2	47.1	0.109	0.007
22	5	21.0	40.8	0.136	0.008
23	8	48.0	56.6	0.194	0.009
24	7	19.1	40.6	0.112	0.007

indicated by the analysis of the entire data set, some structuring is present in *H. forsteriana*. With $K = 2$, many individuals from populations 22, 23 and 24 (all located in the southern, mountainous part of the island, Fig. 1) and also some individuals from population 19 (in the north, Fig. 1) either belong to a cluster distinct from the rest of *H. forsteriana* individuals or are substantially admixed (Fig. 3). The presence of two clusters is supported by ΔK, whereas three or four clusters are supported by the $\ln P(X \mid K)$ criterion, apparently because the only suspected hybrid in *H. forsteriana* is then classified into its own cluster. *K* equal to the number of collecting sites did not receive strong support for any data set (data not shown).

The results of the NEWHYBRIDS analysis were in agreement with those from Structure. The same three individuals were classified as hybrids, surprisingly two of them as F2 hybrids (posterior probability, PP > 0.98), whereas one could have been either an F2 hybrid (PP = 0.29) or a backcross to *H. belmoreana* (PP = 0.66). It seems thus reasonable that these three individuals represent early generations of hybrids. Interestingly, only one of these hybrids was present in a site where both parental species were sampled.

Using Arlequin, we did not detect any evidence for isolation by distance in either species ($P_{Hb} = 0.281$, $P_{Hf} = 0.095$, Mantel test, 10 000 permutations). Although most individuals from the smaller cluster within *H. forsteriana* were located in populations in the south, populations consisting of individuals classified exclusively to the other cluster were present there as well. In contrast, individual-based analyses in Spagedi revealed weak, but significant isolation by distance in both species: *H. belmoreana*, slope $b = -0.0064 \pm 0.0013$ and *H. forsteriana*, $b = -0.0120 \pm 0.0019$. The mean kinship within samples (F_1) was 0.053 ± 0.0068 for *H. belmoreana* and 0.066 ± 0.0083 for *H. forsteriana* suggesting neighbourhood sizes of 148 individuals for *H. belmoreana* and 78 individuals for *H. forsteriana*. These analyses assumed $F_{IS} = 0$ but results were very similar with $F_{IS} = 0.2$. The density of *H. belmoreana* is approximately 10^5 km^{-2} and density of *H. forsteriana* is 6×10^4 km^{-2} in occupied quadrats (5×10^4 km^{-2} and 1.7×10^4 km^{-2}, respectively, over the whole island). Given that effective density may be as little as 10% of census density (Frankham 1995; Vekemans & Hardy 2004), this indicates that effective density is in the range 10^4 to 10^5 adult trees per km^2. The parent-offspring distance, s, can be estimated using $s = (\mathrm{Nb}/4\pi D)^{0.5}$ where Nb is neighbourhood size and *D* is effective density (Rousset 2000). This relationship therefore suggests that *Howea* parent-offspring distance (*s*) is in the range 10–100 m with the difference in slope of the isolation by distance relationship between species largely accounted for by the difference in density. The iterative procedure implemented in Spagedi, which accounts for the expectation that the above relationship only holds over distances in the range *s*–20*s*, failed to converge except for the lowest effective density estimates (largest *s*) probably because the nearest sample sites are separated by around 1 km. The isolation by distance relationship is primarily driven by greater kinship within than between sample sites.

Discussion

Several observations, important in the context of the mode of speciation and evolution of reproductive barriers between palm species, emerge from our genetic analyses.

Geographic structuring of genetic variation in *Howea* species is weak, which is in line with the wind pollination mechanism of *Howea* and small size of the island. The weak isolation by distance and estimated dispersal

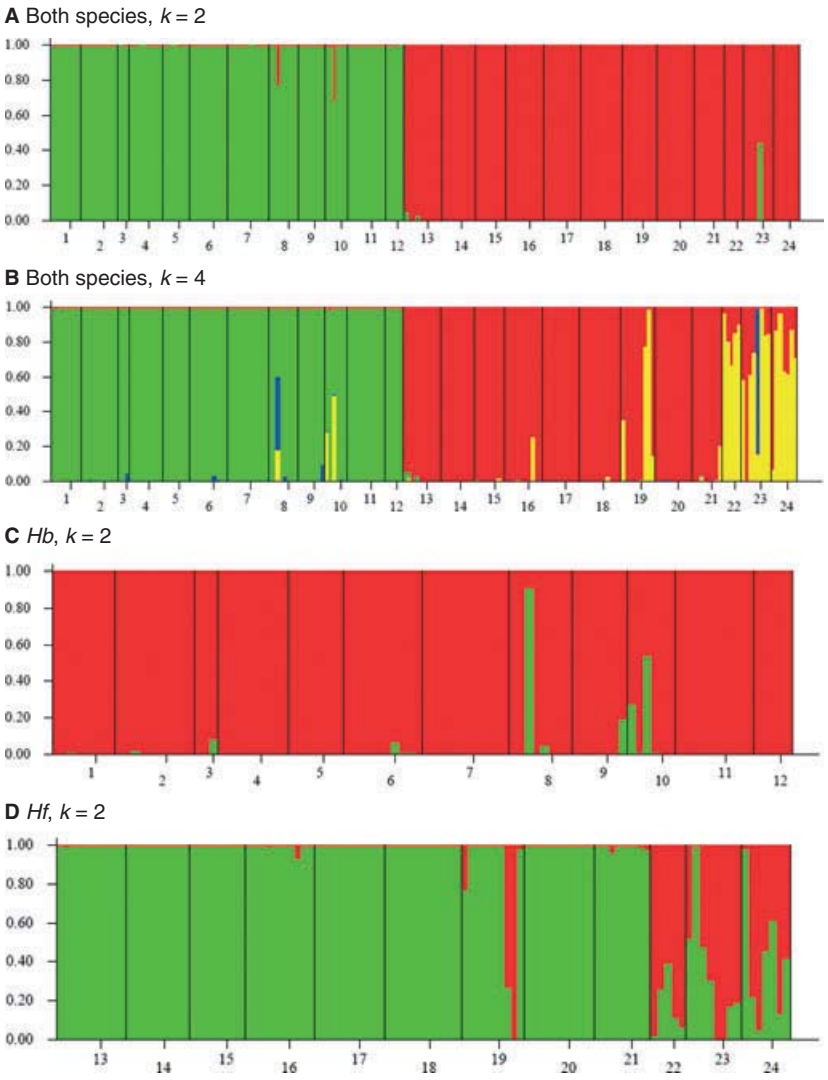

Fig. 3 Results of the Structure analysis. The graphs show the estimated membership coefficients for each individual, in each cluster. Each individual is represented by a single vertical bar, which is partitioned into *K* coloured segments (in proportion to their estimated membership to each of the clusters). (A) Both species, $K = 2$; (B) both species, $K = 4$; (C) *H. belmoreana Hb*, $K = 2$; (D) *H. forsteriana Hf*, $K = 2$.

	Hb + *Hf*		*Hb*		*Hf*	
K	ln *P*(*X* | *K*)	Δ*K*	ln *P*(*X* | *K*)	Δ*K*	ln *P*(*X* | *K*)	Δ*K*
1	−38 825		−11 466		−13 294	
2	−24 636	332.3	−11 649	2.4	−11 924	31.9
3	−25 766	1.4	−11 949	4.3	−11 382	12.0
4	−22 605	18.2	−11 811	2.6	−11 336	1.4
5	−22 963	1.5	−11 692	3.0	−11 347	2.5
6	−23 511		−11 788		−11 345	
10			−11 737		−11 418	
11			−11 584		−11 206	
12			−11 611		−11 316	
22	−24594					
23	−25040					
24	−22995					

Table 4 Results of Structure analysis (*Hb*, *H. belmoreana*; *Hf*, *H. forsteriana*). For a given number of clusters *K*, the table provides ln *P*(*X* | *K*) and Δ*K* (Evanno *et al.* 2005; see Materials and methods). Note that Δ*K* cannot be computed for $K = 1$ or for the highest evaluated number of *K*. *Hb* + *Hf*, both species analysed together; *Hb*, only *Hb*; *Hf*, only *Hf*

(10*s* of metres per generation) probably reflect a balance between long-distance pollen flow and more restricted seed dispersal. Further investigations are needed in this direction. Some population structure was detected in *H. forsteriana*, but individual-based clustering indicates presence of a substantial admixture of two clusters within populations and admixed populations are present in two distinct geographic areas of the island. This in turn matches the lack of substantial geographic barriers to gene flow in these wind-pollinated species over the area of LHI, a conclusion further confirmed by the weak isolation by distance.

Another striking result emerging from our individual-based Bayesian clustering analyses is the finding of very limited current gene flow between species. Among individuals assigned to either species on the basis of their morphology we detected three apparent hybrids, which appear to be F2s or backcrosses to parental species. The high number of variable AFLP loci, and substantial interspecific differentiation should allow detection of even low levels of admixture, if present. Lack of such a signal indicates that, despite occasional hybridization, introgression is indeed limited to early generations of hybrids and thus probably opposed by strong selection.

As for the genetic diversity, *H. forsteriana* shows slightly higher AFLP variation than *H. belmoreana*, which might be attributed to four populations where admixture of two genetic clusters has been observed. However, present-day AFLP variation data may shed little light on the relative population sizes at the time of speciation. More than 50 000 generations have passed since the initial divergence of *Howea* species (Savolainen *et al*. 2006) and mutation may have restored much variation even if it was initially low in either species. Also, if differentiation of species has been occurring in the face of gene flow, then gene exchange across large parts of the genome could have further blurred any initial differences in the level of variation. To evaluate changes in population size since speciation, multiple genealogical markers amenable to demographic analyses should be used within a coalescent framework (Hey 2006; Kuhner & Smith 2007).

Our quantitative analysis of the abundance of *Howea* species indicates that both species are widespread and abundant on LHI with *H. belmoreana* being more widely distributed than *H. forsteriana*. Similarly, the mean number of individuals per quadrat was substantially higher in *H. belmoreana* than in *H. forsteriana*. An important measure of population status and regeneration potential is juvenile recruitment in local populations and there are striking differences between the species in this respect. In *H. belmoreana* juvenile recruitment was observed in all but one quadrat, whereas in *H. forsteriana* juveniles were absent in about a third of the sites in which adults were present. It is not clear whether lower abundance and lower recruitment in *H. forsteriana* are causally connected. It is possible that the lower recruitment in *H. forsteriana* is an indicator of a genuine problem with population regeneration, although it can also be a result of biological differences between the species. For example, monodominant stands of *H. forsteriana* create a large amount of leaf litter that may inhibit seedling establishment, offering a plausible biological explanation for the observed difference between species. In the context of the speciation scenario proposed by Savolainen *et al*. (2006), it is also possible that *H. forsteriana* is relatively maladapted to the new soil type it would have colonized, hence the low figures for abundance/recruitment. It is also possible, however, that these differences reflect the impact of long-term and widespread seed-extraction for the palm trade. It is unlikely to be a result of predation by introduced rats, a major seed predator of LHI palms, as both species are expected to be equally vulnerable in this respect. To obtain a clear-cut picture of the population viability of *H. forsteriana* and the sustainability of the seed harvest, a population viability analysis should be conducted (e.g. a matrix model based on survival parameters measured over two successive years in natural populations), taking into account seed removal for the nursery and seed predation. Such a modelling approach could give reliable indicators for the sustainability of *H. forsteriana* under different seed harvesting regimes. We also note that, in long-lived organisms such as palms, recruitment can be periodical and still sustain viable populations. In any case, a population viability analysis would also shed light on the comparative fitness of both species on different soil types, which is key to an in-depth understanding of the mechanisms of their speciation. Pollination work as well as further experiments on post- versus prezygotic isolation are also needed.

Nevertheless, the fact that genetic structuring in both species is low, combined with the present-day patterns, indicates a very limited role of spatial separation in the development of reproductive isolation. Nonequilibrium explanations for the lack of structure, like recent expansion and/or admixture are not likely as there is no evidence for substantial recent fluctuations in populations of *Howea*. Thus, as far as we can project present patterns into the past, cryptic genetic structure and microallopatry (Butlin *et al*. 2008) are unlikely to have been important factors in speciation of the *Howea* palms. Interspecific gene flow, although present, seems limited to early generations and no evidence for substantial admixture in either species was detected. It seems that, at the present stage of divergence of *Howea* species, barriers to gene flow are

strong enough to effectively prevent introgression in sympatry. Indeed, the theoretical model of Gavrilets & Vose (2007) predicts that strong reproductive isolation developed quickly during sympatric speciation in *Howea*.

We conclude that *Howea* palms do represent a robust case of sympatric speciation and we are now in the process of disentangling further the precise genes and genomic architecture underlying this system. However, we also note that the evidence for some slight isolation by distance combined with the spatial patchwork of habitat types may indicate that mating between diverging populations can be viewed as not totally random with respect to the place of birth – and therefore under some strict definitions (Gavrilets 2004; Butlin *et al*. 2008; Fitzpatrick *et al*. 2008), speciation in *Howea* might be regarded as parapatric.

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Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Abundance of *Howea* palms (adults and juveniles) in randomly generated quadrats on Lord How Island

Table S2 Presence absence data for adults and juveniles of *Howea* palms in quadrats

Table S3 Quadrats for population analysis (black circles on Fig. 1)