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Research Article

Keywords: Demographic history, Divergent natural selection, Dwarfism, Ecotype, Genetic structure, Wind-swept site

Posted Date: June 7th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3019930/v1

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Plant Systematics and Evolution on March 24th, 2024. See the published version at https://doi.org/10.1007/s00606-024-01892-4.

Plant Systematics and Evolution

Dwarfism of *Ficus microcarpa* L.f. in the Ryukyu Islands, Okinawa, Japan

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Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

Short running title: Dwarfism of *Ficus microcarpa*

Abstract

Ficus microcarpa, belonging to Moraceae, is an evergreen tree that can grow on tropical or subtropical rocky coasts. Recently, dwarf *F. microcarpa* individuals have been found on Nakanokamishima Island, Okinawa, Japan, but it remains unclear whether this dwarf trait is based on environmental plasticity or represents an intermediate stage of ecological speciation. To clarify the morphological and genetic traits of dwarfism and consider the process of ecological divergence, we conducted a common garden experiment and performed population genetic and structure analyses using 11 microsatellite markers. Moreover, we conducted a coalescent analysis to estimate the demographic parameters of two genetic clusters: dwarf and tree groups. Common garden experiments clearly classified the samples into two groups: dwarf and tree. In the STRUCTURE analysis, the highest ΔK value was obtained when $K = 2$, suggesting the existence of two genetic clusters: A and B. All samples collected on Nakanokamishima Island were classified into cluster B. Most samples from the other islands were classified into cluster A. Additionally, it was found that dwarf and tree lineages had diverged from an ancestral population hundreds or thousands of years ago. These results indicate that the dwarfism of *F. microcarpa* can be considered an ecotype defined as the intermediate stage of ecological speciation, and that dwarf individuals diversified very recently from an ancestral population with the existence of gene flow.

Keywords

Demographic history, Divergent natural selection, Dwarfism, Ecotype, Genetic structure, Wind-swept site

Introduction

Coastal ecosystems are characterized by severe environmental stresses such as wind exposure, dryness, high light intensity, salt spray and soil salinity. To cope with these conditions, coastal plants often develop morphological and physiological traits including succulent tissues to store water, a pubescent epidermis, a thick cuticle to reduce transpiration and water loss, and dwarfism to withstand strong wind (Hesp 1991; Du and Hesp 2020). Acquisition of these traits allows not only coastal plants but also inland plants to grow in coastal environments. In fact, many studies have reported the invasion and colonization of inland plants that have acquired these adaptive traits for coastal environments (Lowry et al. 2009; Ohga et al. 2013; Liu et al. 2016; Riefner 2016; Itoh 2021).

The process by which inland plants adapt to coastal environments is recognized as a process of ecological speciation via divergent natural selection (Schluter 2000; Nosil 2012; Matsubayashi and Fujiyama 2016). Because selective pressure is counterbalanced by gene flow, and levels of gene flow are often negatively correlated with geographical distance (isolation-by-distance), stronger environmental differences are generally required in sympatric or parapatric conditions for ecological divergence. Here, ecotype or ecological race is defined as the intermediate stage of ecological speciation (i.e., local adaptation), in which several morphological and physiological traits and allele frequencies across loci are grouped despite a lack of intrinsic postzygotic reproductive isolation (Claussen 1951; Kawecki and Ebert 2004; Lenormand 2012; Lowry 2012). Clarification of the factors, processes, and conditions of ecological divergence is one of the major objectives of ecology and evolution.

Researchers have long used common garden experiments to identify the existence of coastal and inland ecotypes across multiple species around the world (reviewed in Lowry 2012). In addition, population genetic and phylogenetic tools are widely used to resolve the patterns and processes of genetic differentiation of ecotypes in plants (Foster et al. 2007; Kokubugawa et al. 2010; Mitsui and Setoguchi 2012; Hirao et al. 2019; Sakaguchi et al. 2020). For example, Hirao et al. (2019) revealed the ecotypic divergence of an alpine herb, *Potentilla matsumurae*, from fellfield to snowbed habitats across a series of mountain sky islands in Japan. Similarly but more locally, Sakaguchi et al. (2020) studied alpine (dwarf) and lowland populations of *Solidago minutissima* on Yakushima Island in Japan, and showed that the dwarf populations have not exchanged genes at significant levels after population divergence, probably because of life-history traits related to dwarfism. At the geographical scale, Mitsui and Setoguchi (2012) studied two sets of riparian/non-riparian lineages of *Ainsliaea* (Asteraceae) over the Japanese Archipelago, and revealed their unique evolutionary and demographic histories.

Ficus macrocarpa L.f. (Moraceae) is an evergreen tree with glossy leaves that is often used as an ornamental plant throughout the world. In tropical and subtropical natural rainforests

and urban cities in Mediterranean regions, this species reaches 10–30 m in height with a wide crown (Polunin 1988; Tan et al. 2010; Yang et al. 2014). However, *F. microcarpa* shows shrubby traits on rocks and cliffs along coasts, on floodplains and banks of tidal rivers, along the edges of swamps, and on other trees as an epiphyte (Polunin 1988; Jim and Chen 2010; Tan et al. 2010; Riefner 2016). In our previous study (Mizutani et al. 2021), we reported that all individuals of *F. macrocarpa* showed obvious dwarfism on Nakanokamishima Island, Okinawa, Japan. Because this island (length: 1550 m, width: 300 m, altitude: 102 m a.s.l.) is characterized by vertical cliffs and steep slopes and all plants except *F. macrocarpa* are herbs, we speculated that the severe coastal environment has contributed to the evolution and/or establishment of dwarf individuals on it. However, it remains unclear whether these dwarf traits are based on environmental plasticity within the species, a parapatric distribution of different species, or an intermediate stage of ecological speciation (e.g., ecotype).

The aims of this study were to clarify the morphological and genetic traits of a putative ecotype (dwarfism) in *F. microcarpa* and investigate the process of ecological divergence in this coastal region. First, we conducted a common garden experiment to examine the genetic background of dwarfism. Second, we conducted population genetic and structure analyses to determine the patterns and levels of genetic differentiation in *F. microcarpa*. Third, we conducted a coalescent analysis to estimate the demographic parameters (e.g., effective population size, divergence time, and migration rates) of two genetic clusters: dwarf and tree groups. Based on the results obtained, we consider the origin and maintenance mechanisms of dwarfism in *F. microcarpa*.

Materials and Methods

Target species and study sites

Ficus microcarpa, with the common names Chinese or Malayan banyan, Indian laurel and curtain fig, is an evergreen tree up to 30 m in height with dangling aerial roots that develop into columnar stems (Polunin 1988; Tan et al. 2010; Chan et al. 2017). Leaves are alternate, simple, leathery, glossy green and oval-elliptic with slightly pointed tips (Polunin 1988; Chan et al. 2017). The tiny flowers grow inside a round, 8–12 mm wide structure called a syconium or fig. Its pollinator is a fig wasp (*Eupristina verticillata*), which has a complicated life cycle intertwined with the flowering of *F. microcarpa*.

The geological distribution of *F. microcarpa* stretches from South (India and Sri Lanka), Southeast and East Asia (China, Taiwan and Japan, the Ryukyu Islands), through Australia, to the Pacific Islands (Tan et al. 2010; Ferrer-Gallego and Boisset 2017). Although *F. microcarpa* is commonly planted in parks and along roads as a shade tree, its natural habitats are riverbanks and tidal floodplains that lie just inland of mangroves, and exposed rocky coasts (Polunin 1988). It is

often seen in old buildings and rock walls around urban areas (Jim and Chen 2010).

This study was conducted on four islands, including Nakanokamishima Island, where dwarf *F. microcarpa* trees were found in a previous study (Mizutani et al. 2021), and three representative islands (Iriomote Island, Ishigaki Island and Okinawa Island) included in the Okinawa Islands and Yaeyama Islands in Okinawa, Japan (Fig. 1). These islands have an oceanic subtropical climate, and their average annual temperature and annual precipitation in 2021 ranged from 23.5℃ to 25.0℃ and from 1500 mm to 2500 mm, respectively (Japan Meteorological Agency). The coasts of Okinawa Island and Ishigaki Island are mainly composed of limestone, but the coast of Iriomote Island contains sandstone and mudstone in addition to limestone. Nakanokamishima Island, the smallest of the four islands, consists only of sandstone and mudstone, and its coastline has suffered severe coastal erosion. The three islands Okinawa Island, Ishigaki Island and Iriomote Island are inhabited islands with diverse vegetation, but Nakanokamishima Island is uninhabited. Only 32 species of seed plants have been recorded on Nakanokamishima Island, and the entire island is covered with grasslands (Mizutani et al. 2021). Nakanokamishima Island supports breeding colonies of six seabird species (Kohno et al., 1986). To protect these breeding seabirds, the Japanese Government designated the island as a national monument in 1972 and as a wildlife protection area in 1981. Therefore, landing and sampling activities on the island are strictly regulated by law.

Common garden experiments

We collected 47 branch samples from the four islands, with 14, 17, 6, and 10 samples from Nakanokamishima, Iriomote, Ishigaki, and Okinawa Islands, respectively. Sampling was conducted for putative natural individuals growing on the coast; individuals that seemed to be artificially planted on the roadside or in hedgerows were avoided. Branch samples were put in a plastic bag with water and were immediately used to make cuttings. Rooted cuttings were transplanted into pots (15 cm in diameter) filled with potting compost (Japan Garden Center Unit Inc., Tokyo). All pots were placed in the field of Shonan Campus of Tokai University (35° 21′ N, 139° 16′ E, 60 m a.s.l.) in Kanagawa, Japan.

We measured seven morphological traits of the samples: relative leaf length, leaf apex shape, leaf thickness, leaf area, internode elongation of branches, number of branches and angle of branches. For the four leaf-related traits, 15 leaves were collected from each individual grown in the field for 6 months from May to November and used for measurements. For relative leaf length, the average value of the ratio of leaf length to leaf width was calculated. For leaf apex shape, the proportion (%) of shapes (acuminate and/or cuspidate) at the tip was determined. The average values of leaf thickness (mm) and area (mm^2) were calculated. For the three branch-related traits, branches from plants grown in the field for 6 months from May to November were

used for measurements. For internode elongation of branches, the average value of multiple internodes elongated during the cultivation period was measured. The number of branches that grew during the cultivation period was counted. For angle (°) of branches, the average value of the angle of the side branches from the main axis was calculated.

We conducted principal component analysis to identify morphological groups: dwarf and tree. The first two principal components (PC1 and PC2) were plotted. Then, the statistical significance of each trait between the groups was examined by a *t*-test at $p < 0.05$.

Genotyping

We collected 173 leaf samples (one leaf from each ramet) from putatively natural individuals including those used for common garden experiments: 26, 61, 54, and 32 from Nakanokamishima, Iriomote, Ishigaki, and Okinawa Islands, respectively. Leaf samples were stored in an airtight container with silica gel at room temperature (20–25°C). Genomic DNA was isolated from samples using the cetyl trimethyl ammonium bromide (CTAB) extraction procedure (Stewart and Via 1993). Polymerase chain reaction (PCR) amplification was performed using a TaKaRa PCR Thermal Cycler Dice Gradient (Takara Bio Inc., Otsu, Shiga, Japan) with 11 nuclear microsatellite markers: LMFC15, 23, 32, 36 (Giraldo et al. 2005), Car8, 9, 11 and Micr3 (Garcia et al. 2012), V183, 188 (Fu et al. 2017) and FT14 (Zhang et al. 2016). Two-step multiplex PCR was performed using universal primers for fluorescent labeling of PCR fragments (Blacket et al. 2012) together with PIG-tailed reverse primers to promote adenylation (Brownstein et al. 1996). In the first step, PCRs were carried out in a final volume of 5.0 μL containing the following: 1 μL of extracted DNA, 2.5 μL of 2× Type-it Multiplex PCR Master Mix (QIAGEN, Hilden, Germany), 0.5 μL of forward primers (final 0.06 μM for each locus) tailed with a specific sequence (universal tail A, B, C, and D), 0.5 μL of reverse primers (final 0.24 μM for each locus) with a pigtail sequence (i.e., GTTTCTT), and 0.5 μL of water. The first amplification was performed under the following conditions: 95°C for 5 min, 18 cycles of 30 s at 95°C, 90 s at 60°C, and 30 s at 72°C, followed by 4°C for holding. Subsequently, 0.5 μL of 2× Type-it Multiplex PCR Master Mix (QIAGEN) and 0.5 μL of fluorescently labeled universal primers (final 0.2 μM for each color: 6-FAM, NED, PET, or VIC) were added for the second step. The second amplification was performed using the following conditions: 6 cycles of 30 s at 95°C, 90 s at 60°C, and 30 s 72°C, followed by 30 min at 60°C. PCR products were analyzed using an Applied Biosystems 3500 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) and fragment sizes were determined with GeneMapper 4.0 analysis software (Thermo Fisher Scientific) and the GeneScan 500 LIZ Size Standard (Thermo Fisher Scientific).

Population genetic analysis

We calculated the number of alleles (N_a), effective number of alleles ($N_e = 1 / \sum P_i^2$, where P_i is the frequency of *i*th allele), observed heterozygosity (H_o), expected heterozygosity (H_e), and fixation index (F) using the GenAlEx 6.5 software (Peakall and Smouse 2006). Null allele frequencies were estimated with Micro-Checker 2.2.3 (van Oosterhout et al. 2004). Genetic differentiation among the four islands was estimated with pairwise *F*st (Peakall et al. 1995) and *G*′st (Hedrick 2005) values using the GenAIEx 6.5 software (Peakall and Smouse 2006) and then tested for statistical significance against 9,999 permutations.

Bayesian clustering was conducted using the STRUCTURE 2.3.4 software (Pritchard et al. 2000) with assumptions of an admixture model and correlated allele frequencies with 100,000 burn-in steps and 100,000 Markov chain Monte Carlo (MCMC) steps. We investigated the likelihood of various numbers of clusters, K = 1–12, with 20 independent runs per K. The support for different values of K was assessed from the likelihood distribution (mean Ln P(K)) and ΔK values (Evanno et al. 2005) produced by Structure Harvester (Earl and vonHoldt 2012).

Isolation-with-Migration (IM) model of lineage divergence and demography

We estimated the demographic history of the dwarf and tree groups under the IM model using the program IMa2 (Hey 2010). To reduce the computation time and to avoid the effects of spatial genetic structure, we selected genetically pure samples (membership coefficient of STRUCTURE analysis (Q) ≥ 0.9) collected from geographically adjacent islands: i.e., 26 samples from Nakanokamishima Island (putative dwarf lineage) and 42 samples from Iriomote Island (putative tree lineage). A series of preliminary runs were conducted to find a suitable heating scheme and prior boundaries of parameters. For the final analysis, MCMC simulations were conducted for 1.0×10^7 steps from which genealogies were saved every 100 steps (i.e., 1.0×10^5 genealogies saved) using a geometric heating scheme (h1 = 0.975, h2 = 0.75) and 24 Metropolis-coupled chains with a burn-in period of 3×10^6 steps. To verify convergence upon the same parameter values, we ran this analysis five times using different random seeds. Then, 3.0×10^5 of the genealogies were sampled with even spacing among all genealogies, and the maximum likelihood estimates (MLEs) and highest posterior density (HPD) of six model parameters were estimated. Here, population size parameters, θ, of the dwarf (d), tree (t) and ancestral (A) lineages are θ_d, θ_t, θ_A, respectively, migration rate parameters, m, from the tree lineage to the dwarf lineage (d) and the dwarf lineage to the tree lineage (t) are m_d and m_t, respectively, and the divergence time parameter between the tree lineage and dwarf lineage from an ancestral population is t. We conducted likelihood ratio tests by comparing the nested demographic models (no migration and equal population size) to the full model (significant migration and different population size) to examine whether population size parameters and migration rate parameters of the dwarf and tree lineages were significantly different. Lastly, estimated model parameters were converted to demographic

quantities (N, m, 2Nm, T) assuming a mutation rate (V) of 0.0005/gene/generation (Estoup et al. 2002; Sun et al. 2009) and a generation time (G) of 50 years; i.e., effective population size is N = θ/4V, migration rate per gene per generation is m = mV, effective migration rate per generation is 2NM = θm/2, and time since ancestral population splitting in year is T = tG/V.

Results

Morphological traits

Common garden experiments showed the existence of a genetic background for the morphological differences observed in *F. microcarpa*. The first component (PC1) of principal component analysis positively contributed to relative leaf length, proportion of leaf apex and internode elongation of branches, and negatively contributed to leaf thickness and angle of branches (Table 1). The second component (PC2) positively and negatively contributed to leaf area and number of branches, respectively. PC1 and PC2 explained 49.2% and 18.4% of the total variance, respectively (Table 1), and the samples were clearly classified into two groups: dwarf and tree (Fig. 2). All samples collected from Nakanokamishima Island were classified into the dwarf group, and most of the samples collected from the other three islands were classified into the tree group. One exception was a sample collected from Iriomote Island, which was classified into the dwarf group.

The dwarf and tree groups showed significant differences in five of the seven morphological traits: relative leaf length, leaf apex shape, leaf thickness, internode elongation of branches, and angle of branches (Table 2). The dwarf group had thicker, more rounded leaves than the tree group, and was characterized by small branch elongation and spread of branches. The tree group had oblong leaves with acuminate and attenuate tips, and the elongation rate of branches was three times higher than that of the dwarf group.

Population genetic structure

All 173 samples were successfully genotyped for 11 microsatellite loci with no missing alleles. The number of alleles (N_a), effective number of alleles (N_e), observed and expected heterozygosity (*H*o and *H*e), and fixation index (*F*) averaged over the four islands (mean ± SE) was 7.6 ± 0.7, 3.9 ± 0.4, 0.64 ± 0.04, 0.64 ± 0.04, and −0.005 ± 0.018, respectively. The estimates of null allele frequencies averaged over the four islands ranged from −0.037 to 0.036 with a mean of −0.007.

There was no apparent difference in genetic diversity estimates among the four islands; Nakanokamishima Island, which is characterized by a small area and dominated by dwarf individuals, showed a slightly lower but generally comparable amount of genetic diversity compared with the other three islands (Iriomote, Ishigaki, and Okinawa) (Table S1). For example, the effective number of alleles (*N*e) and expected heterozygosity (*H*o) on average were 3.04 and

0.606 on Nakanokamishima Island and 3.97–4.45 and 0.639–0.651 on the other islands, respectively. Genetic differentiation among islands was relatively weak but tended to be stronger between Nakanokamishima Islands and other three islands (Table S2). The mean *F*st values between Nakanokamishima and the other three islands and among the latter were 0.045 (range 0.036–0.056) and 0.013 (range 0.012–0.015), respectively. Similarly, the mean *G*′st values between Nakanokamishima and the other three islands and among the latter were 0.165 (range 0.129–0.210) and 0.038 (range 0.033–0.043), respectively. In the STRUCTURE analysis, the highest ΔK value was obtained when $K = 2$ suggesting the existence of two genetic clusters: A and B. All samples collected on Nakanokamishima Island were classified into cluster B with a membership coefficient (Q) of more than 0.9 (Fig. 3). For the other three islands, most samples were classified into cluster A. However, some samples from these islands showed an admixed genetic composition, and four and two samples from Iriomote Island were classified into cluster B at a threshold of $Q \geq 0.9$ and 0.8–0.9, respectively. The STRUCTURE analysis showed that genetic cluster B had undergone significant genetic drift away from an assumed common ancestral cluster; F values were 0.018 and 0.154 for cluster A and cluster B, respectively.

Figure 4 shows the relationship between morphological traits (PC1 values) and genetic compositions (Q of cluster A). Fifteen dwarf individuals (14 from Nakanokamishima Island and one from Iriomote Island) were clearly classified into genetic cluster B. In contrast, tree individuals showed a broad range of genetic compositions: the number of samples for which Q of cluster A was 0.0–0.2, 0.2–0.8, and 0.8–1.0 was 1, 8, and 23, respectively. In summary, the two genetic clusters were relatively undistinguishable compared with morphological traits, but both traits were fixed on Nakanokamishima Island.

Lineage divergence and demography

Table 3 summarizes the maximum-likelihood estimates (MLEs) and 95% highest posterior density (HPD) of the IMa2-derived model parameters; θ_A, θ_t, θ_d, m_d, m_t, and t. Comparison of nested models (no migration and equal population size) to the full model (significant migration and different population sizes) showed that the population size parameters of the dwarf, tree, and ancestral lineages were significantly different: $\theta_d < \theta_t < \theta_A$ ($P < 0.001$). In addition, there was a significant amount of migration between the tree lineage and dwarf lineage (m_d and m_t were greater than zero at $P < 0.001$), and the migration rate from the dwarf lineage to the tree lineage (m_t) was significantly greater than that from the tree lineage to the dwarf lineage (m_d) ($P = 0.011$). Unfortunately, the wide range of these estimates made quantitative description of demographic parameters difficult. For example, the MLE and 95% HPD of effective migration from the tree lineage to the dwarf lineage ($2N_dM_d$) were 0.018 and 0.00–59.3, and those from the dwarf lineage to tree lineage ($2N_tM_t$) were 0.678 and 0.03–90.9, respectively. However, it was clearly shown

that the dwarf and tree lineages had recently diverged from an ancestral population; the MLE of divergence time in years (T) was 700 with a 95% HPD of 300–8,500.

Discussion

The present study showed that (1) *Ficus microcarpa* individuals could be morphologically classified into dwarf and tree groups, (2) genetic differentiation among islands was weak but two population genetic clusters that corresponded to the morphological groups were clearly identified, (3) Nakanokamishima Island and the other three islands (Iriomote, Ishigaki, and Okinawa Islands) were dominated by dwarf and tree individuals, respectively, and most importantly, (4) the dwarf and tree lineages diverged from an ancestral population hundreds or thousands of years ago with the existence of gene flow. These results indicate that dwarfism of *F. microcarpa* can be considered an ecotype, and dwarf individuals diversified very recently from an ancestral population with the existence of gene flow.

The Ryukyu Islands are classified into South, Middle, and North Ryukyu Islands, based on a historical land connection about 200–40 thousand years ago (Kimura 2000). At that time, Nakanokamishima Island, Iriomote Island, and the Ishigaki Islands (i.e., the South Ryukyu Islands) were connected with continental Asia including Taiwan Island, but separated from Okinawa Island (i.e., the Middle Ryukyu Islands). The South Ryukyu Islands were separated from continental Asia about 40–20 thousand years ago. The emergence of the present islands within the South Ryukyu Islands was later than 20 thousand years ago. Here, the divergence time of the dwarf and tree lineages was estimated to be 300–8,500 years ago. While it is difficult to specify the accurate area and time of this divergence event, it is reasonable to conclude that the dwarf individuals growing in Nakanokamishima Island originated within the South Ryukyu Islands.

Several studies have reported the divergence times of ecotypes in different ecosystems and at different geographical scales. For example, the divergence time of the riparian *Ainsliaea linearis* endemic to Yakushima Island in the southern part of the Japanese archipelago and *A. apiculata* distributed across the Japanese archipelago was estimated to be about 25,000 years ago (Mitsui and Setoguchi 2012). Two other riparian species, *A. oblonga* endemic to Okinawa Island and *A. macroclinidioides* widely distributed across the Ryukyu-Taiwan island chain (Mitsui and Setoguchi 2012) diverged about 9,000 years ago (Mitsui and Setoguchi 2012). Within Yakushima Island, the divergence time of alpine dwarf and lowland populations of *Solidago minutissima* was estimated to be 22,628 years ago (Sakaguchi et al. 2020). The estimated time of divergence between dwarf and tree lineages in the present study is comparable but tends to be more recent compared with those studies. Because ecotypic divergence occurs under a balance of gene flow and environmental selection, rapid divergence can be ascribed to either restricted gene flow or strong selective pressure.

It is well known that fig wasps, the pollinators of figs or *Ficus* species, can contribute to long-distance pollen flow (> 100 km) and homogenize genetic structure at the geographical scale (Ahmed et al. 2009; Kobmoo et al. 2010; Heer et al. 2015; Tian et al. 2015; Wang et al. 2018). For example, high genetic diversity and weak genetic structure was reported in *F. sarmentosa* var. *henri* populations (mean H_o = 0.619, mean F_{st} = 0.108) (Wang et al. 2018) and *F. insipida* (mean H_o = 0.58, mean F_{st} = 0.006) (Heer et al. 2015). In the present study, the genetic diversity within islands and genetic differentiation among islands for *F. microcarpa* are comparable to these studies; mean H_o = 0.64 (range 0.61–0.65) and mean F_{st} = 0.102 (range 0.033–0.210). Because the geographical distance between Nakanokamishima Island and Iriomote Island is only 15 km, ecotypic divergence of *F. microcarpa* could not be ascribed to restricted gene flow.

Ecotypes often originate from a single evolutionary event (Levin 2004) and, therefore, the population genetic diversity of ecotypes can be low if a small number of founders maintains the population without effective gene flow from the ancestral population (Le Corre and Kremer 1998; Luikart et al. 1998; Jacquemyn et al. 2020). For example, coastal populations of *Mimulus guttatus* showed consistently lower genetic variation than inland populations (Lowry et al. 2008). Similarly, coastal dune populations of the terrestrial orchid *Epipactis helleborine* derived only recently from inland populations, and current levels of population genetic diversity are low and not related to population size or spatial isolation (Jacquemyn et al. 2020). However, the dwarf population of *F. microcarpa* on Nakanokamishima Island showed a comparable level of genetic diversity compared with the tree populations on the other three islands. This result further supports our speculation that the dwarfism of *F. microcarpa* is maintained under relatively extensive gene flow together with strong environmental selection.

Dwarf individuals of *F. microcarpa* are characterized by thick, round leaves with small branch elongation, which is consistent with morphological traits of other coastal ecotypes (reviewed in Lowry 2012). Nakanokamishima Island is characterized by vertical cliffs and steep slopes, and plants growing on this island would be exposed to strong wind and salt spray especially in the typhoon season. In addition, of 32 species of seed plants in 22 families, *F. microcarpa* is the sole tree species (Mizutani et al. 2021). Taken together, these factors suggest dwarfism plays an important role in the establishment and population maintenance of tree species on Nakanokamishima Island. However, this does not exclude the possibility of dwarf individuals existing on the other islands. In the present study, one individual collected from Iriomote Island showed clear dwarfism in our common garden experiments. In addition, several individuals were classified into genetic cluster B (dwarf cluster) and many individuals showed genetic admixture between the two clusters (dwarf and tree clusters) but had the tree morphology. While plant dwarfism is caused by various mechanisms (Sun et al. 2020) and little is known about the genetic

factors controlling shoot architecture in trees (Hollender et al. 2016; Hill and Hollender 2019), sporadic observation of dwarfism in *F. microcarpa* indicates the existence of recessive dwarfism allele(s) even in the tree populations. This consideration may be important to explain the asymmetric gene flow from the dwarf lineage to the tree lineage and the genetic admixture observed in the morphologically tree group; because recessive traits can be masked by dominant alleles, neutral SSR loci of the dwarf lineage could introgress into the tree lineage more frequently than in the other direction.

To our knowledge, this is the first study to report the dwarfism of fig trees (*Ficus* L.) from the viewpoint of ecotype or local adaptation. While taxonomic identification or phylogenetic classification is outside the scope of the present study, the morphological traits of *F. microcarpa* growing in Nakanokamishima Island seem to be similar to those of *F. microcarpa* var. *crassifolia*, *F. microcarpa* var. *fuyuensis*, and *F. microcarpa* var. *oluangpiensis* distributed on Taiwan Island, about 200 km west of the South Ryukyu Islands (Liao 1989). In addition, dwarf cultivars of *F. microcarpa* have come onto the market, but their origins remain unclear; e.g. *F. microcarpa* cv. Green Mound and *F. microcarpa* cv. Panda (Nagaoka 2020). It would be interesting to consider the genetic relationship between these varieties or cultivars and the dwarf ecotype identified in the present study.

Supplementary Information The online version contains supplementary material available in XX.

Acknowledgements We wish to thank Dr. Zhi-Hui Su of the JT Biohistory Research Hall for his helpful comments on this study and precise identification of *Eupristina verticillata* that pollinate the dwarf *F. microcarpa*. The present study was carried out with permission from the Japanese Ministry of the Environment and the Agency for Cultural Affairs. We thank Robbie Lewis, MSc, from Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript.

Author contributions All authors contributed to the study conception and design. Author YK performed the genotyping and population genetic analysis. Authors MF and AM conducted material preparation and data analyses in the common garden experiment. The first draft of the manuscript was written by authors YK and MF and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability All data and samples (e.g. genotypes, DNA, and leaves) are also available from the corresponding author on reasonable request.

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Figure captions

Fig. 1 Geographic location of Nakanokamishima Island, Iriomote Island, Ishigaki Island and Okinawa Island in the Ryukyu Islands

Fig. 2 Principal component (PC) analysis of 47 individuals based on data for seven traits in the common garden experiment. Each symbol represents an individual collected from Nakanokamishima Island (closed circle), Iriomote Island (open circle), Ishigaki Island (open triangle) or Okinawa Island (open square)

Fig. 3 Genetic structure analyzed using a Bayesian clustering approach in the software STRUCTURE with the optimal delta *K* value ($K = 2$)

Fig. 4 The relationship between the probability of Cluster A (q-value) and principal component (PC1) analysis

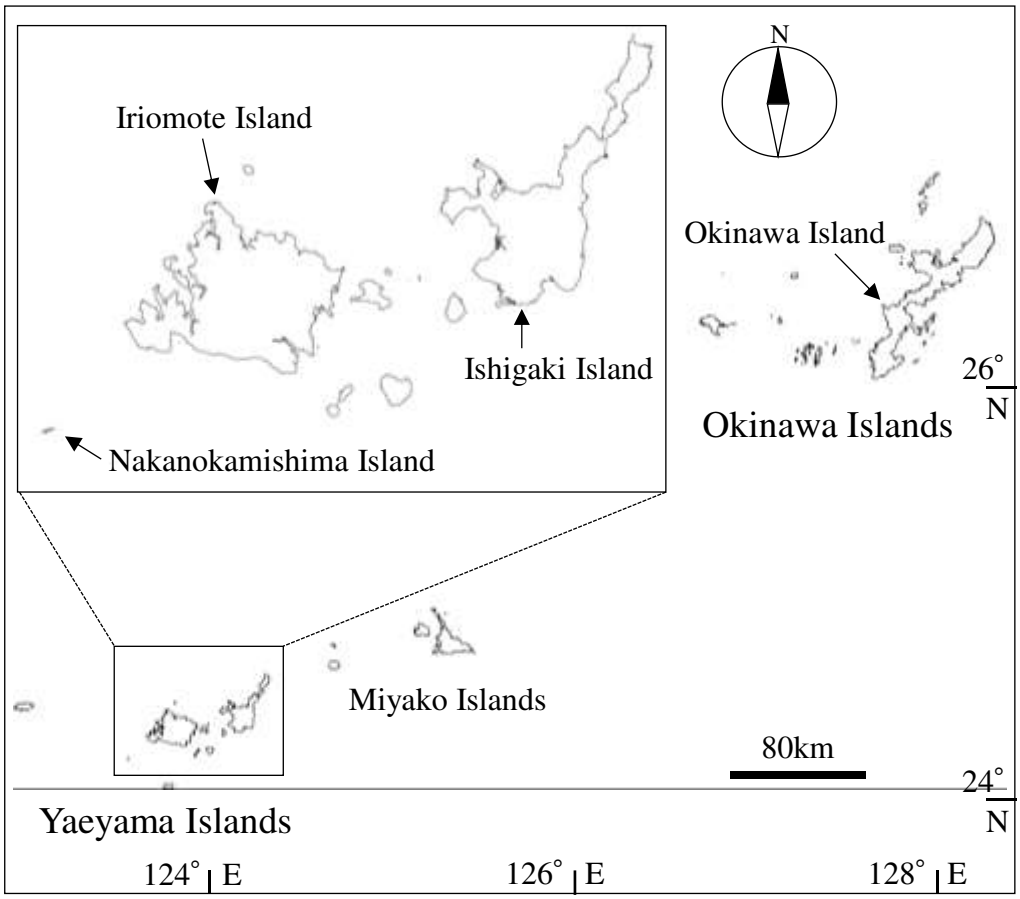
Iriomote Island
Ishigaki Island
Nakanokamishima Island
N
Okinawa Island
Okinawa Islands
26°
N
Miyako Islands
80km
24°
N
Yaeyama Islands
124° E
126° E
128° E

Fig.1

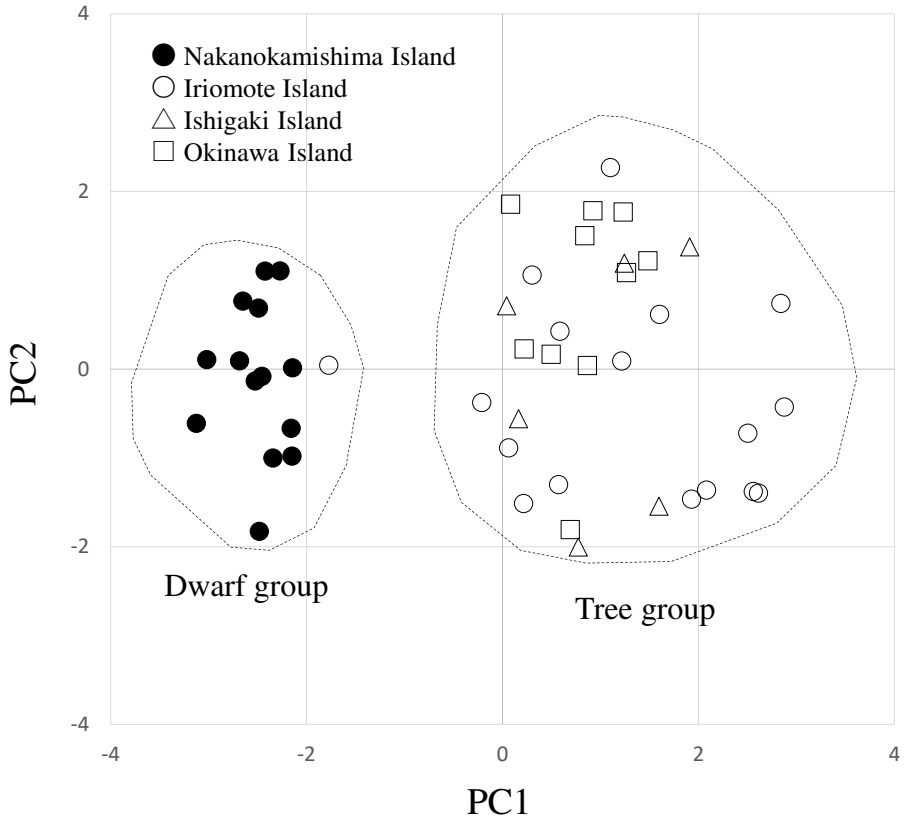
Nakanokamishima Island
Iriomote Island
Ishigaki Island
Okinawa Island
Dwarf group
Tree group
PC1
PC2
4
2
0
-2
-4
-4
-2
0
2
4

Fig.2

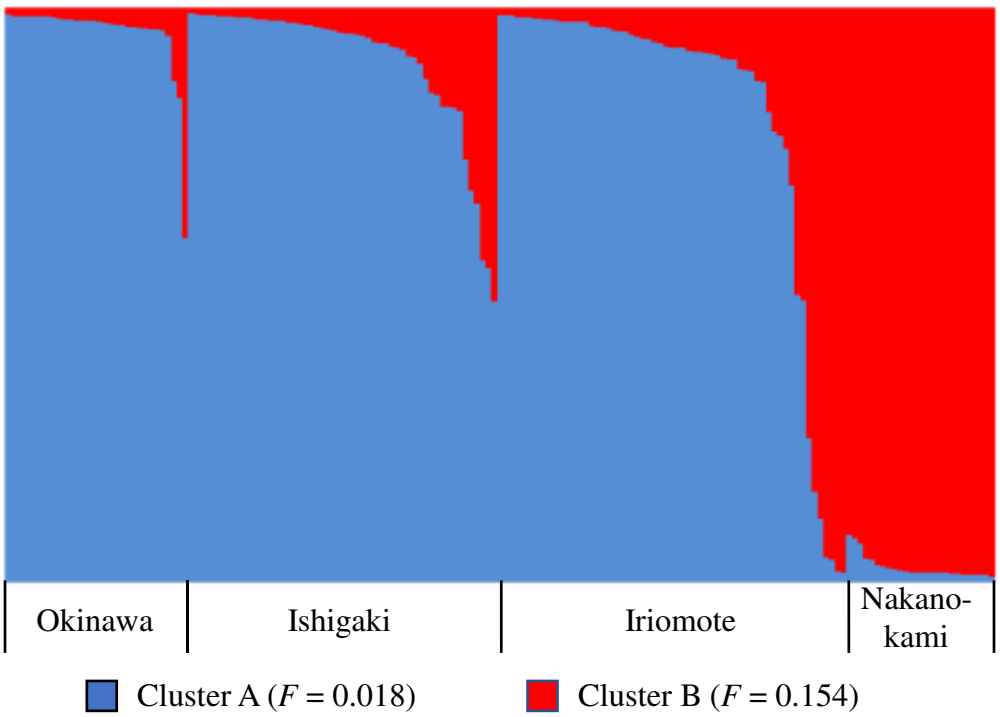
Okinawa
Ishigaki
Iriomote
Nakano-
kami
Cluster A (F = 0.018)
Cluster B (F = 0.154)

Fig.3

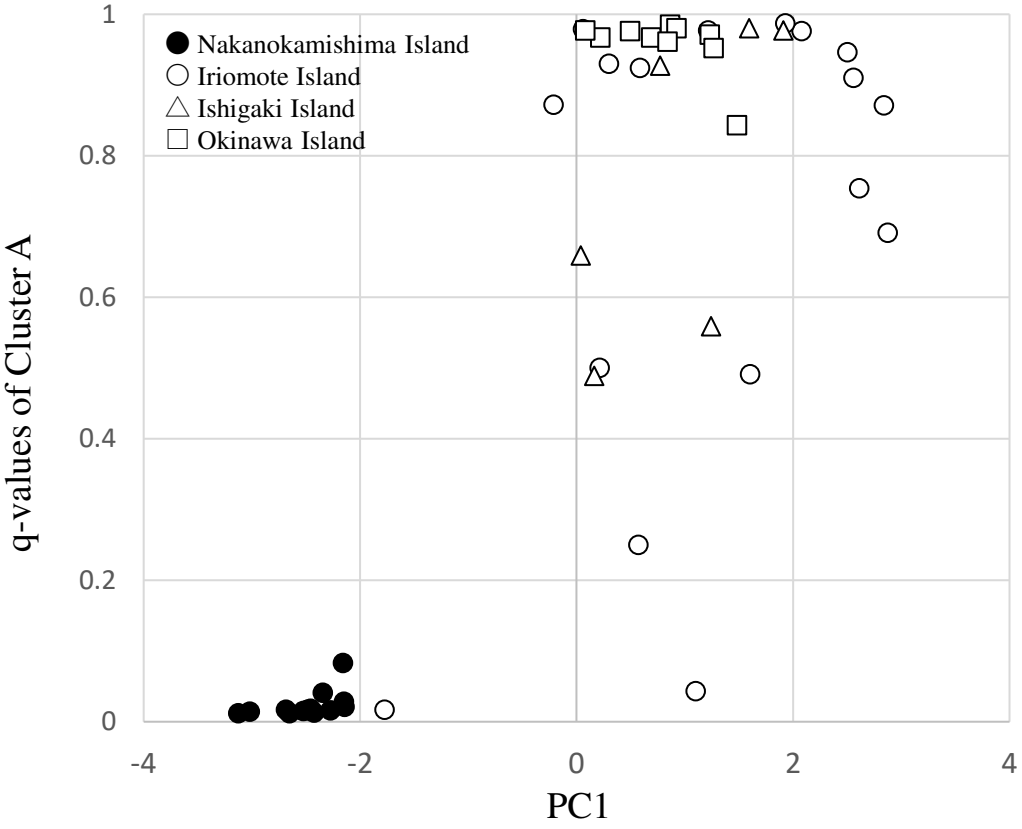
Nakanokamishima Island
Iriomote Island
Ishigaki Island
Okinawa Island
q-values of Cluster A
PC1
1
0.8
0.6
0.4
0.2
0
-4
-2
0
2
4

Fig.4

Table 1 Results of principal component analysis of morphological traits for 47 individuals

	PC1	PC2	PC3	PC4
Contribution(%)	49.2	18.4	11.5	9.7
Traits	Component loadings			
Relative leaf length (leaf length/leaf width)	0.4140	0.1136	-0.3821	0.4776
Proportion (%) of leaf apex shape (acuminate and attenuate)	0.4748	0.1413	-0.1594	0.2905
Leaf thickness (mm)	-0.4562	0.0751	0.1248	0.0628
Leaf area (mm^2 per leaf)	0.0349	0.6919	0.6204	0.2999
Internode elongation (mm) of branches	0.4268	-0.0903	0.4308	-0.3895
Number of branches	0.0755	-0.6882	0.4804	0.5161
Angle (°) of branches	-0.4539	0.0298	-0.1080	0.4190

Table 2 Average values and standard deviation of morphological traits in dwarf type and tree type samples.

	Average and standard deviation per invividual in population	
Traits	Dwarf group (n=15)	Tree group (n=32)
Relative leaf length (leaf length/leaf width)	1.60±0.14 a	1.96±0.22 b
Proportion (%) of leaf apex shape (acuminate and attenuate)	8.9±12.2 a	84.4±22.1 b
Leaf thickness (mm)	0.51±0.04 a	0.41±0.04 b
Leaf area (mm^2 per leaf)	898±219 a	946±218 a
Internode elongation (mm) of branches	3.2±0.9 a	9.1±3.1 b
Number of branches	2.9±1.0 a	3.1±1.4 a
Angle (°) of branches	54±7 a	33±9 b

Table 3 Maximum-likelihood estimates (MLEs) and the 90% highest posterior density (HPD) intervals of model parameters (θd, θt, θA, md, mt, t) from IMa2 analyses. The demographic quantities (Nd, Nt, NA, md, mt, 2Ndmd, 2Ntmt, T) are estimated based on the mutation rate (V) of 0.0005 /gene/generation and the generatio time (G) of 50 years.

	θ_d	θ_t	θ_A	m_d	m_t	t	N_d	N_t	N_A	m_d	m_t	$2N_dm_d$	$2N_tm_t$	T (year)
MLE	0.04	0.12	28.4	0.9	11.3	0.007	20	60	14220	0.0005	0.0057	0.018	0.678	700
HPD95Lo	0.00	0.12	15.2	0.0	0.5	0.003	0	60	7860	0.000	0.0002	0.00	0.03	300
HPD95Hi	0.76	1.4	51.6	156.1	129.9	0.085	380	700	25820	0.078	0.0650	59.3	90.9	8500

Nd, Nt, NA : effective population sizes of the dwarf lineage, the tree lineage, and the ancestral population, respectively. N=θ/4V.

md, mt : migration rate from the tree lineage to the dwarf lineage and the dwarf lineage to the tree lineage, respectively (per gene per generation). m=mV.

2Ndmd, 2Ntmt : effective migration rate from the tree lineage to the dwarf lineage and the dwarf lineage to tree lineage, respectively (per generation). 2NM=θm/2

T : time since ancestral population splitting (in year). T=tG/V

Table S1 Genetic diversity of *Ficus microarpa* in four islands. Values are shown as the mean ± 1 standard error per locus based on the variation at 11 microsatellite loci.

	N	*N*a	*N*e	*H*o	*H*e	*F*
Okinawa	32	7.18 ± 1.21	3.97 ± 0.72	0.679 ± 0.077	0.639 ± 0.075	- 0.065 ± 0.036
Ishigaki	54	9.18 ± 1.62	4.45 ± 0.86	0.645 ± 0.083	0.648 ± 0.080	0.010 ± 0.035
Iriomote	61	9.27 ± 1.55	4.31 ± 0.79	0.614 ± 0.075	0.651 ± 0.079	0.056 ± 0.017
Nakanokami	26	4.64 ± 0.49	3.04 ± 0.36	0.615 ± 0.070	0.606 ± 0.060	- 0.018 ± 0.044

N, number of genets; *N*a, number of alleles; *N*e, effective number of alleles; *H*o, observed heterozygosity; *H*e, expected heterozygosity; *F*, fixiation index.

Table S2 Genetic differentiation of *Ficus microarpa* across four islands (lower diagonal: pairwise *F*st; upper diagonal: pairwise *G*'st).

	Okinawa	Ishigaki	Iriomote	Nakanokami
Okinawa	0.000	0.033	0.043	0.210
Ishigaki	0.013	0.000	0.038	0.157
Iriomote	0.015	0.012	0.000	0.129
Nakanokami	0.056	0.042	0.036	0.000

All values were significant ($P < 0.001$).