RESEARCH PAPER

Neither insects nor wind: ambophily in dioecious *Chamaedorea* palms (Arecaceae)

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ABSTRACT

Pollination of Neotropical dioecious trees is commonly related to generalist insects. Similar data for non-tree species with separated genders are inconclusive. Recent studies on pollination of dioecious *Chamaedorea* palms (Arecaceae) suggest that species are either insect- or wind-pollinated. However, the wide variety of inflorescence and floral attributes within the genus suggests mixed pollination mode involving entomophily and anemophily. To evaluate this hypothesis, we studied the pollination of *Chamaedorea costaricana*, *C. macrospadix*, *C. pinnatifrons* and *C. tepejilote* in two montane forests in Costa Rica. A complementary morphological analysis of floral traits was carried out to distinguish species groups within the genus according to their most probable pollination mechanism. We conducted pollinator exclusion experiments, field observations on visitors to pistillate and staminate inflorescences, and trapped airborne pollen. A cluster analysis using 18 floral traits selected for their association with wind and insect pollination syndromes was carried out using 52 *Chamaedorea* species. Exclusion experiments showed that both wind and insects, mostly thrips (Thysanoptera), pollinated the studied species. Thrips used staminate inflorescences as brood sites and pollinated pistillate flowers by deception. Insects caught on pistillate inflorescences transported pollen, while traps proved that pollen is wind-borne. Our empirical findings clearly suggest that pollination of dioecious *Chamaedorea* palms is likely to involve both insects and wind. A cluster analysis showed that the majority of studied species have a combination of floral traits that allow for both pollination modes. Our pollination experiments and morphological analysis both suggest that while some species may be completely entomophilous or anemophilous, ambophily might be a common condition within *Chamaedorea*. Our results propose a higher diversity of pollination mechanisms of Neotropical dioecious species than previously suggested.

INTRODUCTION

Dioecious plants (*i.e.* with separated genders) comprise nearly 6% of angiosperm species and are distributed over 7% genera of flowering plants (Renner & Ricklefs 1995). Pollination of dioecious plants is commonly attributed to generalist insects but approximately 30% of dioecious genera, mostly in temperate regions, are wind-pollinated (Bawa 1980; Renner & Ricklefs 1995).

In tropical humid forests, palms (Arecaceae family) are conspicuous elements of the vegetation (Dransfield *et al.* 2008) and are predominantly composed of monoecious and hermaphroditic species; however, the dioecious genus *Chamaedorea* Willd. stands out as the largest Neotropical palm group, with 107 species (Govaerts *et al.* 2012). Knowledge of the pollination systems in *Chamaedorea* has relied on observations of flower visitors and floral morphology that tentatively assigned species to an entomophilous (insect) or anemophilous (wind) system (see references in Henderson 1986). Empirical studies on the pollination of *Chamaedorea* are relatively recent and

only comprise around 6% of all the species. Results thus far have tended to categorise species as either entomophilous or anemophilous. Anemophily has been suggested for *Chamaedorea pinnatifrons* (Jacq.) Øerst. (Listabarth 1993), *Chamaedorea alternans* H. Wendl. (Otero-Araiz & Oyama 2001) and *Chamaedorea radicalis* Mart. (Berry & Gorchoy 2004), while entomophily has been suggested for *C. ernesti-augusti* H. Wendl., *C. oblongata* Mart., *C. neurochlamys* Burret and *Chamaedorea tepejilote* Liebm. (Porter-Morgan 2007).

Floral attributes and inflorescence architecture within *Chamaedorea* are associated with either wind or insect pollination. Species show bright or indistinctly coloured petals, dry-powdery or moist and sticky pollen, unscented or scented flowers with varying amounts of anatomical protection, and spicate or paniculate inflorescences with erect or pendulous rachillae (Hodel 1992a; Askgaard *et al.* 2008). A combination of these floral traits within a single species is likely to allow successful pollination by both wind and insects.

The diversity in floral morphology within the genus suggests a combination of pollination modes, but previous experimental

work classified *Chamaedorea* species as either entomophilous or anemophilous. Listabarth (1993) observed small thrips (Thysanoptera) and beetles (Coleoptera) visiting staminate inflorescences of *C. pinnatifrons*, but insects were rarely seen in pistillate flowers. Listabarth (1993) proposed that the insect's activity facilitated the release of pollen from flowers where wind could more easily transport it to pistillate flowers, in a system he named 'insect-induced wind pollination'. Otero-Arnaiz & Oyama (2001) also classified *C. alternans* as wind-pollinated, however reported activity of thrips on staminate inflorescences. Conversely, Porter-Morgan (2007) argued that thrips were the main pollinator of four *Chamaedorea* species and gave wind a secondary role. These authors, however, did not consider the possibility of a mixed pollination syndrome.

The simultaneous occurrence of insect and wind pollination, or ambophily, is documented in several angiosperm families, such as Ericaceae, Euphorbiaceae, Polemoniaceae, Ranunculaceae and Salicaceae (Faegri & van der Pijl 1979; Culley *et al.* 2002; Duan *et al.* 2009; Yamasaki & Sakai 2013). Within Arecaceae, this system has been documented in seven species (Barfod *et al.* 2011). In ambophilous plants, the role of pollination vectors varies, from species predominantly insect-pollinated to mostly wind-pollinated species, with most species showing intermediate frequencies (Culley *et al.* 2002).

In this study, we aimed to understand the role of wind and insect pollination in four *Chamaedorea* species. The genus is a diverse group with a broad geographic distribution, from Central Mexico to Bolivia, and is more abundant and diverse in the understorey of middle elevation forests between 800 and 1500 m (Hodel 1992a). Most species are restricted to the Mesoamerican region, where two centres of diversity are recognised: southern Mexico–Guatemala and the mountains of Costa Rica and Panama (Hodel 1992a; Henderson *et al.* 1995).

Our specific research goals were to: (i) experimentally evaluate the contribution of insect and wind pollination to pistillate reproductive success of four *Chamaedorea* species in Costa Rica (ii) describe the pollination mechanism associated with entomophily; and (iii) identify potential entomophilous and anemophilous groups within the genus, as well as groups with a combination of the two pollination modes, inferred through a morphological analysis of floral traits on a sample of 52 *Chamaedorea* species.

MATERIAL AND METHODS

Study species and sites

The selected species, *Chamaedorea costaricana* Øerst., *Chamaedorea macrospadix* Øerst., *C. pinnatifrons* and *C. tepejilote*, have three to six (eight) pinnate leaves per stem; branched, racemose inflorescences with several rachillae; small (<5 mm), greenish to yellow, scented flowers; dry-powdery pollen; and black fruits at maturity (Hodel 1992a). *C. costaricana* has multiple stems up to 8-m high and occurs from southern Mexico to western Panama, from 500 to 2350 m a.s.l.; *C. tepejilote* develops a single erect (rarely multiple) stem 5-m high and occurs from southern Mexico to Colombia, from 0 to 1600 m a.s.l.; *C. macrospadix* has an initially acaulescent habit but typically later forms a short stem and is restricted to Costa Rica and western Panama, from 100 to 1500 m a.s.l.; *C. pinnatifrons* develops single stems to 4-m high and has the widest

geographic distribution, from southern Mexico to Bolivia in South America, from (100) 400 to 2600 m a.s.l. (Hodel 1992a; Grayum 2003). Voucher specimens were deposited at the herbaria CR and USJ (Cascante 995, 996, 1166; Ríos 16, 21, 22, 23).

Our study was conducted in two humid tropical montane forests of the Central Valley of Costa Rica. El Rodeo forest is located in the western part of the valley (9°54'25"N, 84°16'30"W, 900–1000 m a.s.l.). It is a premontane wet forest (*sensu* Holdridge 1967) on hilly terrain where *C. costaricana*, *C. macrospadix* and *C. tepejilote* grow sympatrically. El Rodeo experiences a marked seasonality: a dry season from December to March, with mean annual precipitation of 2467 mm and mean annual temperature of 23.4 °C (Instituto Meteorológico Nacional undated). All three *Chamaedorea* species were located within two 1-ha permanent plots established to monitor forest dynamics (Cascante-Marín *et al.* 2011). Our second site, La Carpintera forest (9°53'20" N, 83°58'10"W) is a lower montane moist forest at 1750 m a.s.l. in Cartago Province. The forested area, mostly old secondary growth, covers nearly 2400 ha and is frequently covered with cloud (Sánchez *et al.* 2008). La Carpintera experiences a mild dry season from January to April, with mean annual precipitation of 1563 mm and mean annual temperature of 16.6 °C (Instituto Meteorológico Nacional undated). *C. pinnatifrons*, which is relatively abundant and the only palm species in the understorey at La Carpintera, flowers from February to June.

The role of insects and wind in pollination

We implemented conventional pollinator exclusion experiments on pistillate inflorescences and compared fruit set (*i.e.* ratio of fruits to flowers) per inflorescence to control inflorescences (Kearns & Inouye 1993). We performed the experiments during two flowering seasons, 2005–2006 and 2010–2011 at El Rodeo and 2011 and 2012 at La Carpintera. A total of 112 pistillate inflorescences of *C. costaricana*, 56 of *C. macrospadix*, 131 of *C. pinnatifrons* and 141 of *C. tepejilote* were distributed among the following pollination treatments: (i) insect exclusion using white mesh cloth bags with pores 150–200 µm in size and sprayed with domestic insecticide (active ingredient: pyrethroids) to deter insect visitation but that allowed wind-borne pollen to enter (pollen size 20–25 µm) (ii) control inflorescences without any manipulation and exposed to wind and insect pollination; and (iii) total exclusion using paper and plastic bags to test for apomixis. Bags covered the inflorescences for 6–8 weeks after anthesis to reduce fruit loss due to predation. Fruits were counted when they had reached nearly one-third of their final size.

Kruskal–Wallis and Mann–Whitney tests with a Bonferroni correction ($K = 3$, $P = 0.0167$) were used to compare fruit set among treatments per species. All analyses were performed using R 2.15.1 (R Core Development Team 2012).

Biotic pollination vectors

We recorded insect visitors from inflorescences of both genders of all species. Visitation frequency of insects on pistillate inflorescences was surveyed twice a week in the flowering period of 2011–2012 in a sample of 164 pistillate inflorescences of *C. costaricana*, 82 of *C. macrospadix*, 140 of *C. pinnatifrons* and 159 of *C. tepejilote*. We collected insects by placing the inflorescence

at anthesis in a plastic bag and gently shaking it to detach insects, which were preserved in 70% ethanol for later identification by a specialist.

Because preliminary observations indicated that thrips (Thysanoptera) were the most abundant flower visitors, we assessed pollen loads on thrips visiting pistillate flowers from five inflorescences of each species (one in *C. macrospadix*). All thrips from each pistillate inflorescence were placed in separated vials with 1 ml 70% ethanol. In the laboratory, the insects were mounted on glass slides and examined using a light microscope at the highest magnification (1000 $\times$) to count pollen grains on their bodies. The ethanol solution was also checked for pollen grains that may have detached from the insects. Then, we estimated the mean number of grains per thrip by considering the total number of pollen grains and thrips per vial.

To evaluate the putative role of staminate inflorescences as brood sites (*sensu* Sakai 2002), we collected thrips from staminate inflorescences in anthesis and senescence (4–7 days after anthesis). Thrips were counted and classified according to their development stage (*i.e.* adults or juveniles: instar I to pupae). We sampled 23 inflorescences (12 in anthesis and 11 in senescence) of *C. tepejilote*, 27 (14 and 13) of *C. costaricana*, and 15 (nine and six) of *C. macrospadix* during the 2010–2011 flowering season, and 16 (eight and eight) inflorescences of *C. pinatifrons* during the 2011 season.

We also investigated if insects assisted the release of pollen from the anthers in species with staminate flowers with apically connate petals (*sensu* Listabarth 1993). We bagged five staminate inflorescences near anthesis of *C. macrospadix* and *C. pinatifrons* with transparent plastic bags. These bags excluded thrips and were placed before insects visited inflorescences. The enclosed inflorescences were periodically checked during anthesis to determine if pollen was spontaneously released from the anthers and accumulated outside the flowers or on the inner bag surface.

Abiotic pollination vectors

Traps to collect wind-borne pollen were placed in four randomly selected stations in the understorey of both study sites in 2010–2011 (only 2011 for La Carpintera). Each pollen trap consisted of a microscope slide coated (*ca.* 2 cm²) with melted fuchsin stained glycerol-gelatin on one side (Kearns & Inouye 1993). Each station contained three traps hanging down from vegetation at 1.5–1.8 m above the ground. Pollen traps were separated by at least 1 m from each other and were left in place for 3–4 days. New traps were set every 3–4 days at El Rodeo and weekly at La Carpintera. Pollen grains from *Chamaedorea* were identified and counted with a light microscope using a reference collection. *Chamaedorea* pollen is readily identifiable; however, because pollen morphology and size are comparable among all four studied species, it was not feasible to identify pollen to the species level.

Floral diversity and pollination syndromes within *Chamaedorea*

To estimate the prevalent pollination syndromes within the genus, we surveyed 18 discrete floral traits grouped according to inflorescence morphology, which included attributes related

to aerodynamic properties of wind-borne pollen (Niklas 1992; Friedman & Harder 2004), flower attributes and pollen type traits from 52 *Chamaedorea* species. We tallied the presence/absence of characters commonly associated with the wind or insect pollination, according to theoretical syndromes following Faegri & van der Pijl (1979) and Proctor *et al.* (1996). Information on floral traits was obtained from Hodel (1992a) and additional sources: Douglas (1988), Hodel (1992b, 1995), Hodel *et al.* (1995), Grayum (1998, 2003), Otero-Arnaiz & Oyama (2001), Berry & Gorchov (2004), Porter-Morgan (2007), Askgaard *et al.* (2008), and notes from herbaria specimens deposited at the National Herbarium of Costa Rica (CR), National Institute of Biodiversity (INB), Herbarium of the University of Costa Rica (USJ) and personal field observations.

Similarity among species was calculated using the simple matching index (Krebs 1999) for binary data. Then, a cluster analysis was carried out to group species according to their floral similarity, which putatively reflects a particular pollination syndrome, using the unweighted paired group method (UPGMA; Legendre & Legendre 2012). In order to identify the floral traits responsible for the differences among groups generated by the cluster analysis, we carried out a similarities percentages (SIMPER) analysis using the Bray–Curtis index (Clarke & Warwick 1994). These analyses were performed using PAST, version 2.16 (Hammer *et al.* 2001).

RESULTS

The role of anemophily and entomophily on fruit set

Fruit set under the control treatment (*i.e.* inflorescences exposed to wind and insects) ranged from 27% in *C. pinatifrons* and 34% in *C. macrospadix*, to 50–55% in *C. costaricana* and *C. tepejilote* (Fig. 1). In all four studied species and reproductive years, the control treatment produced consistently more fruits than the insect exclusion treatment alone (all *P*-values <0.001 after Mann–Whitney test). Fruit set from insect-excluded inflorescences varied among species and flowering seasons, from nearly 7% in *C. pinatifrons* to 22% in *C. tepejilote* (Fig. 1). Within species, fruit set from wind pollination was significantly higher than fruit set from apomixis in most cases (Mann–Whitney test *P* < 0.001, except in *C. tepejilote* in 2005–2006 and *C. macrospadix* in 2011 *P* > 0.05). Pollen caught on pollen traps confirmed that wind transports *Chamaedorea* pollen (Fig. 2). A mean of 86 $\pm$ 11 ($\pm$ SD) pollen grains per trap was counted at El Rodeo, while at La Carpintera, we found 16 $\pm$ 4 grains per trap. Fruit development by putatively apomictic means was detected in all but one species (*C. pinatifrons*), but varied among species and flowering seasons (Fig. 1), and overall it was <11%. Seed viability tests on apomictic fruits were not performed.

Thrips as biotic pollinators

Thrips were the most frequent and abundant flower visitors of all studied species, particularly on staminate inflorescences, at both sites (Fig. 3). In a single staminate inflorescence, we counted more than 2000 individuals belonging to *Brooksithrips chamaedoreae* (Thripidae), a species described from our study site of El Rodeo (Retana-Salazar & Mound 2005). Thrips were

Fig. 1. Fruit set under different pollination treatments in *Chamaedorea costaricana*, *Chamaedorea tepejilote*, *Chamaedorea macrospadix* and *Chamaedorea pinnatifrons* (black bars) control treatment (grey bars) insect exclusion and (white bars) insect and wind exclusion (apomixis). Flowering seasons of (A): 2011 (B): 2012 at La Carpintera and (C): 2005–2006 and (D): 2010–2011 at El Rodeo, Costa Rica. Data are mean fruit set per inflorescence. Vertical bars are 1 SE. Letters represent significant differences between treatments per species after a Mann–Whitney test (Bonferroni correction, $P = 0.0167$).

seen visiting 12% of the pistillate inflorescences of *C. costaricana*, 9% of *C. macrospadix*, 24% of *C. pinnatifrons* and 26% of *C. tepejilote*. Infrequent visitors to staminate inflorescences

Fig. 2. Temporal distribution of *Chamaedorea* wind-borne pollen during (A): the 2010–2011 flowering season at El Rodeo, and (B): the 2011 season at La Carpintera, Costa Rica. Data from four stations at El Rodeo and four stations at La Carpintera for 14 and five sampling dates, respectively. The average grains per trap $\pm$ SE (vertical lines) is indicated.

were the stingless bees *Trigona fulviventr* and *Plebeia* sp. at El Rodeo and *Partamona orizabaensis* (Apidae: Meliponini) at La Carpintera. These insects were not recorded visiting pistillate inflorescences. Other visitors on *C. pinnatifrons* staminate inflorescences were staphylinid, chrysomelid (*Demotista* sp., *Tetragonotes* sp.) and cryptophagid beetles. Both *Tetragonotes* sp. and the unidentified cryptophagid mated in staminate inflorescences, but were rarely seen on pistillate flowers.

Thrips used staminate inflorescences of the four *Chamaedorea* species as brood sites, although we also observed juvenile thrips on pistillate inflorescences, but this was an uncommon event ($n = 3$). Adult thrips fed on pollen, mated and laid eggs on the adaxial face of the petals. It is noteworthy that in *C. tepejilote*, thrips not only fed on pollen but also preyed upon the tissue of the inflorescence rachis (Fig. 3). After a few days, we observed juvenile thrips on senescent staminate inflorescences (Fig. 3, Table S1).

A total of 259 *Chamaedorea* pollen grains from 221 thrips were quantified on pistillate inflorescences of *C. tepejilote*, 212 grains from 200 thrips on *C. costaricana* and 400 grains from 224 thrips on *C. pinnatifrons*. Thus, on average, each thrips transported 1.0 ± 0.6 grains on *C. tepejilote*, 1.1 ± 0.7 on *C. costaricana* and 2.5 ± 2.9 on *C. pinnatifrons*; no pollen was found on the nine thrips caught from the *C. macrospadix* inflorescence.

Thrips are not required for pollen release from the anthers of flowers with apically connate petals of *C. pinnatifrons* and *C. macrospadix*. We observed pollen deposited outside the corollas and between flowers a few days after anthesis on inflorescences where insect visitation was prevented.

Fig. 3. (A): A staminate inflorescence of *Chamaedorea tepijilote* (Arecaceae) overcrowded with thrips (B): the same inflorescence a few days later showing thrips damage on rachis (white dotted areas on the rachis are immature thrips) (C): pistillate flowers of *C. tepijilote* visited by thrips (D): an adult *Brooksithrips chamaedoreae* (Thysanoptera) (E): immature thrips indicated by arrows on staminate flowers of *C. tepijilote*. Line scales: A to C = 10 mm, D = 5 mm, E = 2 mm.

Floral diversity and pollination syndromes within the genus

Five groups were identified in the cluster analysis on floral traits, with an overall average dissimilarity of 49.7% (Fig. 4). The inflorescence and floral traits that contributed more to separating groups included the length of the inflorescence peduncle, the number of rachillae, staminate flower showiness and anther position; these traits explained 53.1% of the dissimilarity. The traits with the lowest contribution to distinguishing groups included the texture of the rachis, pollen type, presence of scented staminate flowers and position of pistillate rachillae; these traits explained 10.5% of the differences.

Noteworthy, none of the groups were entirely described by anemophilous or entomophilous traits alone; instead, most species possessed a combination of inflorescence and flower characteristics adapted for wind as well as insect pollination. Species in groups 1 and 3 (Fig. 4) have a prevalence of traits more conducive to wind and insect pollination, respectively. In group 1, species exhibited inflorescences with long peduncles (traits C and D), many rachillae (traits G and H), flowers not showy (traits K and L), exposed and divided stigmas (traits M and N) and exerted anthers (trait O); these traits are congruent or suggest anemophily. In contrast, species in group 3 exhibited traits more adapted to entomophily (Fig. 4), e.g. inflorescences with short peduncles, few rachillae, showy flowers, entire and inserted stigmas and inserted anthers.

Species in the other groups (2, 4 and 5; Fig. 4) showed different ranges of combinations of entomophilous and anemophilous floral traits, either at the inflorescence or flower level. Three of our study species (*C. costaricana*, *C. tepijilote* and *C. pinnatifrons*) were placed among species with a combination of floral traits (group 2), while *C. macrospadix* was placed in group 1, an anemophilous group.

DISCUSSION

Our empirical findings from pollination experiments show that wind and insect pollination co-occur in our four studied species. Floral trait analyses suggest that pollination mechanisms within the dioecious palm genus *Chamaedorea* do not constitute a dichotomy between entomophilous and anemophilous species, since most species exhibit a combination of floral traits for both insect and wind pollination. While some *Chamaedorea* species may be completely entomophilous or anemophilous, most analysed species have floral traits that simultaneously allow for the transport of pollen by both vectors, a system termed ‘ambophily’ (reviewed in Culley *et al.* 2002). These results suggest that based on morphological traits, a large number of species may be ambophilous. This type of mixed pollination should be explicitly considered in future experiments of pollination biology in *Chamaedorea*.

Our field experiments suggest that thrips (Order Thysanoptera) mainly carry out entomophilous pollination in the studied *Chamaedorea* spp. However, the role of other visiting insects cannot be discounted because pollination studies in the literature barely include 6% of all *Chamaedorea* spp. Also, other insect groups might pollinate species with wide geographic distribution or those found in diverse habitats (Herrera *et al.* 2006). Thrips have been considered unreliable pollinators due to their limited flight ability and their apparently low specialisation for hosts (Kirk 1985a, 1997), but they appear to be effective pollinators in our study sites.

The thrips pollination system in *Chamaedorea* fits the brood-site type of ‘pollen parasites’ described by Sakai (2002) and discussed by Porter-Morgan (2007). Insects are probably attracted to the scented staminate flowers (Kirk 1985b) even before anthesis, where they mate and feed on pollen and plant

Fig. 4. (Right) UPGMA of 52 *Chamaedorea* species based on inflorescence and flower traits related to wind and insect pollination. (Left) Shade matrix graph for the same species showing the scoring of the analysed traits. Dark areas represent the character state associated with insect pollination. Letters refer to inflorescence and flower traits (see description in Appendix A). Characters with the lowest contribution are not shown (after the SIMPER analysis, see text). The species in the present study are underlined.

tissue and oviposit within the small flowers. Most of the 52 *Chamaedorea* species analysed (96%) had scented staminate flowers. Consumption of vegetal tissue was more evident on staminate inflorescences, particularly in *C. tepejilote*; this consumption might be related to a low amount of anatomical defences. The increasing number of juvenile thrips found on drying staminate inflorescences indicates that immature insects develop on senescent flowers within a few days. Later, juvenile insects probably fall to the soil to complete their development (Lewis 1973). We found that thrips indeed transported pollen on their bodies, and volatile compounds (mainly sesquiterpenoids in *C. tepejilote*; Kaiser 1997) probably attract them to the pistillate flowers. Nonetheless, in our floral trait analysis only 31% of the species had scented pistillate flowers, which suggests that if thrips are the main pollinators of most of the analysed *Chamaedorea*, they also might locate pistillate flowers through visual cues.

Pollen deposition onto the stigma of pistillate flowers of *Chamaedorea* is likely mediated by a 'mistake mechanism'

(Dafni 1984). Pistillate flowers are morphologically similar to their staminate counterparts and imitate the oviposition substrate. However, they do not provide a brood site inside the small pistillate flowers with reduced perianth parts and lack pollen as food. Also, and although not studied here, septal nectaries to provide nectar as reward have not been detected in flowers of *Chamaedorea* (Askgaard *et al.* 2008). Pollination occurs when thrips arrive at pistillate flowers and do not stay for a long time, explaining the observed lower number of insects on pistillate inflorescence compared to staminate inflorescences. Before leaving, their activity on the inflorescence provides the opportunity for pollen to be deposited on the exposed stigmas and to develop the one-seeded fruit. Alternatively, pistillate inflorescences might inhibit thrips oviposition or larval development due to a possible larger anatomical defences (Uhl & Moore 1973) or active inhibition of larval development (Dufay & Anstett 2004).

Insects, particularly beetles, bees and flies, are generally assumed to pollinate palms. While thrips are occasionally

mentioned as flower visitors, their potential role as palm pollinators has not been fully recognised (Kirk 1997; Barfod *et al.* 2011). Some previous pollination studies in *Chamaedorea* may have overlooked the importance of thrips. For instance, the supposedly anemophilous *C. alternans* (Otero-Arnaiz & Oyama 2001) might also experience entomophily because the authors reported large amounts of thrips on staminate inflorescences. Listabarth (1993) suggested an indirect role of thrips in the pollination of *C. pinnatifrons*; however, our observations show that thrips have a direct role in pollen transport to pistillate flowers. The role of thrips as pollinators is documented in several plant groups with different taxonomic affinities, such as Zamiaceae (Mound & Terry 2001), Annonaceae (Gottberger 1994; Momose *et al.* 1998), Dipterocarpaceae (Appanah & Chan 1981; Kondo *et al.* 2011), Euphorbiaceae (Fiala *et al.* 2011), Moraceae (Sakai 2001; Zerega *et al.* 2004) and Ericaceae (Hagerup & Hagerup 1953; García-Fayos & Goldarazena 2008).

In our study sites, anemophily appears to contribute significantly to the pollination biology of *Chamaedorea*. Initially, palms were considered anemophilous due to their floral morphology (*i.e.* small flowers, high pollen–ovule ratio and small pollen grains), but this view has changed to consider palms as mainly entomophilous (Henderson 1986; Barfod *et al.* 2011); however, several studies have documented the role of insects and wind in palm pollination (Anderson *et al.* 1988; Scariot *et al.* 1991; Consiglio & Bourne 2001; Melendez-Ramirez *et al.* 2004). Our pollination experiments showed that fruit set in the studied *Chamaedorea* species is the result of a mixed pollination system. In addition to thrips, *Chamaedorea* pollen grains can also be wind-transported, as our pollen trapping experiment documented. Pollen release from the anthers in species with apically connate petals, such as *C. pinnatifrons*, might occur by spontaneous means and not necessarily need the intervention of thrips, as Listabarth (1993) suggested. Consequently, for species like *C. tepejilote* and *C. costaricana* with apically separated petals, pollen release into the wind is easily achieved without insect intervention. Pollen movement by wind in *Chamaedorea* can be a common phenomenon, even in the relatively low-wind forest understorey, because the majority (85%) of the species selected for our analysis of floral traits possessed dry and powdery, easily wind-transported pollen.

Combining wind and insect pollination might increase pollen transfer and is most advantageous when the reliability or effectiveness of insect pollinators varies in space and time (Culley *et al.* 2002; Duan *et al.* 2009; Yamasaki & Sakai 2013). Having a dual mixed pollination system is expected to increase the reproductive success of plants (Costa & Machado 2012) and therefore may represent a selective advantage. This enhanced success is particularly important for species in conditions where insect pollinators are scarce or species compete for pollinators (references in Culley *et al.* 2002). The effectiveness of both pollination systems in *Chamaedorea* is also likely to depend on floral traits that are adapted to both wind and insect pollination (Yamasaki & Sakai 2013) and very likely vary according to species, habitat and flowering season (Culley *et al.* 2002). In our study species, wind pollination significantly contributed to overall fruit set. We are however likely to underestimate the role of wind because the bag itself probably reduced somewhat the inflow of wind-borne pollen (Kearns & Inouye

1993). In the ambophilous palm *Attalea speciosa* Mart. ex Spreng. (studied as *Orbignya phalerata* Mart.), anemophily was more important in open sites or pastures compared to forest conditions (Anderson *et al.* 1988). In *Cocos nucifera* L., ambophily is recognised but the relative importance of wind and insect as pollen vectors might depend on wind conditions (Melendez-Ramirez *et al.* 2004). In *Chamaedorea*, ambophily may also contribute to long-distance pollen dispersal because thrips dispersal might be limited (Kondo *et al.* 2011).

Floral trait analysis showed that some *Chamaedorea* species might have flower traits that are congruent with wind pollination, while others have more insect pollination attributes. In our analyses, however, most species showed a combination of characters from both pollination mechanisms, which may favour ambophily. In fact, three of our study species are clustered in a group with species having both anemophilous and entomophilous floral traits. However, *C. macrospadix* was placed by the cluster analysis in a group with floral characters adapted for anemophily, while our pollination experiments showed that insect pollination played an important role. In this species both staminate and pistillate flowers are scented, which may promote insect visitation. In addition, the flowering period of *C. macrospadix* in our study population comprises the second peak of the rainy season, which may constraint the role of wind pollination (data not shown). This also indicates that the classification suggested by our morphological analysis needs to be confirmed empirically through pollination studies.

Several other ambophilous angiosperms show a similar phenomenon of intermediate floral morphology between wind and insect pollination, with some traits typical of wind pollination and other traits primarily of insect pollination (Karrenberg *et al.* 2002; Costa & Machado 2012; Yamasaki & Sakai 2013). However, it remains to be determined whether ambophily in *Chamaedorea* represents a stable reproductive strategy or an intermediate step in the transition to full wind or insect pollination (Culley *et al.* 2002; Friedman & Barrett 2009). It is important to note that we only analysed 52 *Chamaedorea* species, and other pollination groups may arise if traits from all species are studied.

Chamaedorea palms grow in the understorey of undisturbed humid Neotropical forests and represent conspicuous elements of the vegetation at these sites. As dioecious species, *Chamaedorea* palms are more sensitive to the detrimental effects of habitat disturbance, deforestation and fragmentation on breeding dynamics (Somanathan & Borges 2000). Information on the breeding system is necessary for the development of efficient conservation strategies, because they impact gene flow patterns, which in turn affect levels of genetic diversity and population structure (Loveless & Hamrick 1984). This study aimed to increase knowledge about the pollination of this palm group, while trying to elucidate the existence of complex pollination mechanisms. Moreover, our results show a higher diversity in pollination mechanisms of Neotropical dioecious species than previously suggested by other authors (*e.g.* Bawa & Opler 1975; Renner & Ricklefs 1995). We clearly show ambophily as the most likely pollination system for the four studied *Chamaedorea* species in Costa Rica. Future research directions on this topic might evaluate the role of both pollination mechanisms along a habitat or geographical gradient or in isolated populations in forest fragments.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Mean thrips abundance in staminate inflorescences on anthesis and senescence in the four studied *Chamaedorea* species.

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APPENDIX A

List of floral characters used to separate species groups according to their putative pollination modes within *Chamaedorea* palms. Characters were selected and scored following the pollination syndrome hypothesis and aerodynamics of wind-borne pollen (see Material and Methods for details).

Inflorescence morphology

A. Staminate peduncle size: short (<50 cm; 1), long (>50 cm; 0). B. Staminate rachillae position: erect (1), pendulous (0). C. Staminate branching: spicate (1), branched (0). D. Number of staminate rachillae: few (<5; 1), many (>5; 0). E. Staminate rachis: white and soft (1), green and hard (0). F. Pistillate peduncle size: short (<50 cm; 1), long (>50 cm; 0). G. Pistillate rachillae position: erect (1), pendulous (0). H. Pistillate branching: spicate (1), branched (0). I. Number of pistillate rachillae: few (<5; 1), many (>5; 0). J. Pistillate rachis: white and soft (1), green and hard (0).

Flower morphology

K. Staminate colour: showy (yellow or orange; 1), unshowy (green; 0). L. Pistillate colour: showy (yellow or orange; 1), unshowy (green; 0). M. Anthers: inserted (1), exerted (0). N. Stigmas: inserted (1), exerted (0). O. Stigma shape: entire (1), divided (0).

Fragrance

P. Staminate: present (1), absent (0). Q. Pistillate: present (1), absent (0).

Pollen

R. Type: moist and sticky (1), dry and powdery (0).