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Sexual systems in gymnosperms: a review

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Abstract

The aim of this study was to update figures for the presence of dioecy among the gymnosperms and investigate its correlation with climate, growth form, pollination and seed dispersal syndromes, and risk of extinction. Dioecy was found in almost 65% of contemporary gymnosperm species, a higher percentage than previous estimates. It dominates in 8 of the 12 families. As in angiosperms, dioecious gymnosperms are particularly common in climbers and are more commonly found in tropical climates. Analysis of the degree of threat using IUCN red list categories showed that the proportion of threatened species is higher in dioecious than in monoecious species only in temperate climate. The high sensitivity of dioecious species to environmental changes associated with human activity in temperate climate may explain this

phenomenon. The monophyly of extant gymnosperms and the relatively small number of species (about 1000) create the possibility of treating them as a model group in investigating the evolution of sexual systems.

Keywords: Endangered species; Dioecy; Monoecy; Evolution

Introduction

Dioecy is relatively rare in plants. Hermaphroditic species dominate among angiosperms and dioecious species account for around 6% of plants (Renner & Ricklefs, 1995; Weiblen, Oyama & Donoghue, 2000; Renner, 2014). Despite its rarity, dioecy does have its advantages. The most emphasized advantage of dioecy is the complete exclusion of the risk of self-pollination (Darwin, 1876; Charlesworth & Charlesworth, 1978). A second advantage is the optimization of resource allocation to both male and female functions. This is one of the explanations why fleshy, presumably expensive, fruits are more common in dioecious species (Maynard-Smith, 1978; Charnov, 1982; Renner & Ricklefs, 1995).

Nevertheless, it is often emphasized that dioecy is not an optimal sexual system in sedentary organisms. Indeed, hermaphroditism is considered to be the best strategy for optimizing fitness where cross-pollination opportunities are limited since automatic or pollinator-assisted self-pollination is sometimes possible if no other partner is near (Charnov, 1982). A second disadvantage of dioecy is that the number of individuals producing seed is half that of hermaphrodites because seeds are carried only by females. This results in dioecious species having reduced seed dispersal, the so-called seed-shadow handicap (Heilbuth, Ilves & Otto, 2001). Moreover, although female organisms can optimise resource allocation, they often

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expend greater reproductive effort over a longer period of time compared to males (Vessella *et al.*, 2015; Garbarino *et al.*, 2015; Matsushita, Takao & Makita, 2016; Zarek, 2016). It is also known that sexes differ in their response to stress (Juvany & Munné-Bosch, 2015; Ariel & Alejandro, 2016), and sexes can also have varying environmental requirements and occupy slightly different niches, potentially leading to spatial sex segregation (Cox, 1981; Bierzychudek & Eckhart, 1988).

Due to these disadvantages, a hypothesis has emerged that dioecy is evolutionarily less advantageous than hermaphroditism, that is, sporophytes with bisexual strobili or flowers (Westergaard, 1958). Supporting this, it has been shown that within the angiosperms, clades that consist of dioecious plants contain fewer species than their sister clades; this suggested that dioecious species were more prone to extinction and that dioecy was an evolutionary 'dead end' (Heilbuth, 2000). Nevertheless, species diversification rates have been shown to be as high in dioecious as in hermaphrodite lineages (Käfer et al., 2014; Käfer & Mousset, 2014; Sabath et al., 2016). Thus, the primary cause of the lower number of dioecious species in sister clades could be the frequent reversion to hermaphroditism (Käfer et al., 2017; Goldberg et al., 2017), in agreement with reports arguing that such reversions are not infrequent in angiosperms (Barrett, 2013; Renner, 2014).

Dioecy in angiosperms has evolved repeatedly, either from monoecy or from gynodioecy (Charlesworth & Charlesworth, 1978; Renner & Won, 2001; Barrett, 2002). There is also the possibility of the reverse phenomenon, that monoecy can evolve from dioecy, as exemplified in the *Momordica* genus, where it has possibly happened seven times (Schaefer & Renner, 2010). In gymnosperms, dioecy has repeatedly evolved from monoecy, for example, 10 to 13 times just within the Pinopsida (Leslie *et al*., 2013). Gymnosperms do not have gynodioecy nor any of the many other sexual systems from which dioecy can and has evolved

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in angiosperms. Differences in the genetic mechanisms of sex determination in both angiosperms and gymnosperms suggest that there are multiple pathways of the evolution of dioecy. Sex chromosomes are known in only about 40 species of plants, mainly in angiosperms (Ming, Bendahmane & Renner, 2011), however, heterochromosomes in gymnosperms have been found in *Ginkgo biloba* L., where a ZW/ZZ system is present, whereas *Cycas revoluta* Thunb. is characterized by a XX/XY system. Species of *Podocarpus*, such as *P. macrophyllus* (Thunb.) Sweet, *P. longefoliolatus* Pilg. and *P. elatus* Seem. ex Parl., also present a unique if unspecified system of sex determination (Hizume, Shiraishi & Tanaka, 1988; Ming *et al*., 2011); *P. macrophyllus* females have four sex chromosomes ($X_1X_1X_2X_2$) while males have three (X_1X_2Y). On top of any genetic determination, many dioecious species can change sex under the influence of environmental factors.

More research on reproductive syndromes has been carried out on angiosperms than on gymnosperms. It has been shown that dioecy in angiosperms is found more frequently among trees, lianas or shrubs than among herbaceous species (Renner & Ricklefs, 1995; Vamosi, Mazer, & Cornejo, 2008). Dioecy is also more common in tropical climates and islands than in moderate or cold conditions (Bawa, 1980; Baker & Cox, 1984; Vamosi & Vamosi, 2004). Additionally, pollination in dioecious species is frequently by wind rather than insects whereas seed dispersal is by animals rather than wind (Bawa, 1980; Thomson & Brunet, 1990; Charlesworth, 1993; Vamosi, Otto & Barrett, 2003; Schlessman *et al.*, 2014). Accordingly, Givnish (1980) concluded that wind-pollinated and animal-dispersed gymnosperms are usually dioecious, and wind-dispersed gymnosperms are usually monoecious. However, the classification of gymnosperms has recently changed dramatically and there is a need for a review of sexual systems in gymnosperms in the light of the new groupings to see if these generalisations still hold true.

The proportion of gymnosperm species assumed to be dioecious has varied. Thus, Givnish (1980) scored 420 (52%) of 804 gymnosperm species as dioecious. Owens and Hardev (1990) reported that 25% of 680 species were entirely dioecious and another 11% sometimes dioecious, sometimes monoecious. Ming *et al.* (2011) calculated that 36% of 1010 gymnosperms are dioecious, including all Gingkoaceae, Cycadaceae and Gnetaceae, and Kumar, Kumari and Sharma (2014) reported that 36% of 1021 species are dioecious. Here we review the current knowledge about the occurrence of dioecy in gymnosperms and determine whether dioecy is related to climate, growth form, pollination system, strobilus type.

Materials and methods

Data on dioecy were compiled for 1033 species across all gymnosperm families from available literature (Farjon, 2010; Osborne *et al.*, 2012; Ickert-Bond & Renner 2016) and databases such as The International Plant Names Index, The Plant List, World Checklist of Selected Plant Families and The Gymnosperm Database. In addition to sexual dimorphism, data were compiled on the climate in which a particular species is present, method of pollination, seed dispersal mechanism (animal or wind), growth form (Raunkiaer, 1934; De Langhe *et al.*, 1983), and the degree and threat of extinction according to the red list of International Union for Conservation of Nature (IUCN). Statistical analysis was carried out using JMP software (SAS Institute).

Results

In total, 667 of the 1033 (64.6%) species currently accepted (see Appendix A; Table 1) are dioecious, similar to the 52% obtained in 1980 when the number of accepted gymnosperm species was 804 (Givnish 1980). An additional 14 species (*c*. 1%) had mixed systems (i.e.

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dioecious and monoecious). Dioecious species are found within 42 of the 84 genera (see Appendix A). In six of the 12 gymnosperm families, all species are dioecious - Cycadaceae, Ephedraceae, Gingkoaceae, Gnetaceae, Welwitschiaceae and Zamiaceae - and in the Podocarpaceae and Taxaceae, the majority of species are dioecious (94.9% and 93.7%, respectively). A lower proportion of dioecious species occurs in the Cupressaceae (29.6%) and particularly in the Araucariaceae (5.4%). There are no dioecious species in the Sciadopityaceae and Pinaceae (but see two *Pinus* species below). Four families contain species that are either mono- or dioecious. There are eight such species in the Cupressaceae, three in the Podocarpaceae, two in the Pinaceae (*Pinus edulis* Engelm. and *P. cembroides* Zucc. - *P. cembroides* var. *bicolor* (Little) Silba, (sometimes identified as a distinct species - *P. johannis* M.-F.Robert, then Pinaceae has one dioecious species) is almost completely dioecious and one in the Taxaceae (*Taxus brevifolia* Nutt.).

All climbing species, and almost all of the chamaephytes (growing <0.5 m above ground level) and nanophanerophytes (<3 m above ground) are dioecious (97.6 and 90.4%, respectively; Table 2), while among the phanerophytes (>3 m high) dioecious species constitute only 43.9%.

There was a noticeable connection between sexual dimorphism and climate (Table 3). Tropical species are predominantly dioecious, while cooler climate species are mostly monoecious (Table 3). All ambophilous species (pollinated by both wind and insects) are dioecious and almost all dioecious species are dispersed by animals (Table 4). The exception is *Welwitschia mirabilis* the seeds of which are wind-dispersed (see Appendix A). Conversely, dioecious species that are wind-pollinated are frequently animal-dispersed (272 species; 93%). All monoecious species are wind pollinated and 95% are wind dispersed (336 species; Table 4).

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Nearly 95% of gymnosperm species examined are found on the IUCN Red List of Threatened Species: 48 dioecious and 4 monoecious species are not classified because of lack of data. Table 5 shows that dioecious and monoecious species are unequally distributed across categories (Chi^2 test p <0.0001); 50.7% of monoecious species are recorded in the LC (Least Concern) category, while only 33.4% of dioecious species are in this category. The other categories, from NT (Near Threatened) to EW (Extinct in the Wild), include nearly 60% of dioecious and around 48% of monoecious species (Table 5). In the case of the 14 species with a mixed system (mono-dioecious), 11 species are recorded in LC (Table 5). However, Fisher's exact test showed that probability of being a threatened or endangered species (joined: NT - Near Threatened, VU - Vulnerable, EN - Endangered, CR - Critically Endangered, EW - Extinct in the Wild) is greater for dioecious than monoecious species only in temperate climates (Table 6). The climate has a major impact on the degree of threat to the species because the proportion of species threatened and endangered in relation to the "Least Concern" (not threatened) species increases from cold to tropical climate (Table 6).

Discussion

Dioecy is the dominant sexual system in gymnosperms found in 667 of 1033 species (64.6%). It can be seen that there is a significant difference between gymnosperms (where dioecy dominates and there are no hermaphroditic flowers) and angiosperms in which about 6% of the 261,750 total species accepted in the Angiosperm Phylogeny website or 5% of the 304,419 species accepted in The Plant List species are dioecious (Renner, 2014). In 8 out of the 12 families currently in the gymnosperms, dioecy accounts for more than 90% of all species (including six families at 100%). The level of ~65% of dioecy in gymnosperms supports Givnish's (1980) estimate of 52% and rejects the 25% calculated by Owens and

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Hardev (1990) and the 36% calculated by Ming et al. (2011). Partly this is due to increased species numbers; for instance, there are now 107 dioecious species in *Cycas* (Osborne *et al.*, 2012), up from 20 known to Givnish (1980).

Growth form

All 36 climbing gymnosperms are dioecious (Table 2). A similar pattern has been found in angiosperms, where climbing growth was strongly correlated with dioecy (Renner & Ricklefs, 1995). Moreover, a positive correlation between dioecy and woody growth (including climbers, shrubs, trees) results primarily from the association between dioecy and climbing growth in angiosperms (Renner & Ricklefs, 1995). Shrub growth form is more weakly associated with dioecy, while the tree growth form is not associated with dioecy in angiosperms (Renner & Ricklefs, 1995). This is consistent with the trend observed in gymnosperms because dioecy is much more common in chamaephytes and nanophanerophytes (growing point <3 m above ground, mostly shrubs) than in phanerophytes (>3 m above ground, mostly trees): respectively 97.6, 90.4% and 43.9% of species (Table 2).

Climate

The relationship between the climate and the sexual system in gymnosperms is complicated since it is affected by the geographical distribution of families. For example, most species of Pinaceae (203 species) occur in temperate to mild climates, compared to only 34 found in a tropical climate (see Appendix A). However, a connection between sexual dimorphism and the climate can still be seen; the cooler the climate, the fewer the dioecious species (Table 3). Tropical Cycadaceae and Gnetaceae are represented only by dioecious

species, whereas Pinaceae of colder areas have no dioecious species (although there are two species with a mixed system). Overall, 83.0% of tropical gymnosperms are dioecious, falling to 54.7% in temperate areas and 15.6% in cold areas. Dioecy is often connected with a tropical climate (Sakai & Weller, 1999). Moreover, this is not an indirect correlation between the tropical climate and fleshy fruits or woody growth form, since dioecy is independently associated with a tropical climate at the family level in angiosperms (Renner & Ricklefs, 1995). It suggests that dioecious taxa developed when the climate on Earth was warmer (Vamosi *et al.*, 2003). During periods of cooling, these dioecious taxa were progressively lost from colder areas as a result of poorer adaptation to a cool climate.

Pollination and seed dispersal

There is a strong relationship between dioecy and pollination by insects in gymnosperms, because all species pollinated by both wind and insects (ambophily) are dioecious. However, there is no clear answer as to which came first: dioecy or ambophily? Contemporary Pinaceae, which are almost all monoecious, are anemophilous, whereas entomophily is found in families that currently only contain dioecious species (Cycadaceae, Zamiaceae, Gnetaceae, Ephedraceae). Entomophily is not found in extant Pinopsida but was present in the extinct family Cheirolepidiaceae in the Mesozoic (Labandeira, Kvaček & Mostovski, 2007). Pollination of mesozoic *Cycas* species by insects is also known. Such an association developed at the latest in the Cretaceous (Peñalver *et al.*, 2012), but probably earlier (Labandeira, Kvaček & Mostovski, 2007). Ambophily is common in *Cycas* and is found in some living species (Schneider *et al.*, 2002; Kono & Tobe, 2007; Procheş & Johnson, 2009; Terry *et al.*, 2012). In the case of *C. revoluta*, insect exclusion experiments resulted in about 10% seed set, whereas with natural pollination it was 40% (Kono & Tobe, 2007). Even more prominent is the association of *Lepidozamia peroffskyana* Regel (Cycadales) pollinating beetles

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(O. Coleoptera); 70% of ovules were pollinated in the presence of beetles along with wind exclusion, whereas in the absence of beetles the value dropped to just 0.1% (Hall *et al.*, 2004). Perhaps the use of insects is related to the difficulty of carrying pollen through the wind in dense vegetation (Procheş & Johnson, 2009). The entomophily of *Welwitschia mirabilis* Hook.f. has long been known (Pearson, 1909). *Gnetum* L. species are also pollinated by insects (Kato, Inoue & Nagamitsu, 1995), while most of Ephedraceae are anemophilous (Rydin & Bolinder, 2015). It is still an open question whether entomophily was the primary condition in Gnetaceae (Gong *et al.*, 2015) and whether anemophily of *Ephedra* L. species is secondary (Rydin & Bolinder, 2015). The answer may be linked to climate: tropical species are more frequently insect-pollinated than those from colder areas (Bawa, 1980)

Monoecious gymnosperms are primarily dispersed by wind, whereas in dioecious species dispersal by animals is dominant (Givnish, 1980). Animal-dispersed species are found in 97% of dioecious gymnosperms and only in 4.8% of monoecious species. A similar relationship between fleshy fruit and dioecy has also been found in angiosperms (Renner & Ricklefs, 1995), but a significant correlation between dioecy and fleshy fruit was only found in the Eumagnoliids and Asterids, but not in Rosids (Vamosi *et al.*, 2003). The type of cone is related to the seed dispersal: fleshy cones are often carried by animals, whereas dry cones are dispersed by wind. Fleshy seeds and fruits in dioecious plants attract animal seed dispersers and increase the efficiency of dispersal (Geldenhuys, 1993) which can favour the development of stable dioecious populations (Barot & Gignoux, 2004).

Degree of threat

The greater proportion of dioecious gymnosperms threatened with extinction may support the evolutionary deadend hypothesis (Heilbuth, 2000) for dioecious gymnosperms.

However, our study shows a bias because the degree of threat may be associated with other ecological factors within dioecious gymnosperms. Undoubtedly, climate has a major impact on the degree of threat to the species in our study because the proportion of species that are threatened and endangered increases from cold to tropical climate (Table 6). This is confirmed by the number of endangered plant species in North America being positively correlated with annual temperature (Dobson et al, 1997). However, compared with monoecious species, dioecious species are significantly underrepresented in the "Least Concern" category only in temperate climates.. Perhaps the explanation for this phenomenon is the long-standing and intense human disturbance in temperate regions. Agricultural activity is known to be the key negative variable affecting threatened plants (Dobson *et al*., 1997). Dioecious species may be more sensitive to environmental changes (Petry *et al*., 2016; Retuerto *et al*., 2018), which could explain their threatened status in temperate climates.

Evolution of sexual systems in gymnosperms

Dominance of dioecy in gymnosperms is somewhat surprising given that dioecy is rare in sedentary organisms (Charnov, 1982). The lack of hermaphroditic flowers seems to be the key to the prevalence of dioecy in gymnosperms. Perfect flowers are found in more than 80% of angiosperm species (Yampolsky & Yampolsky, 1922; Renner & Ricklefs, 1995). This provides a successful sexual system because angiosperms have well-developed physical and genetic barriers to reduce self-fertilization, including self-incompatibility (SI). SI has been reported in over 100 families and is found in an estimated 39% (database size not stated) of angiosperms species (Igic, Lande & Kohn, 2008). A comparison of over 1500 species belonging to Asteraceae, Brassicaceae and Solanaceae showed that 66% of island and 41% of mainland species were self-compatible (Grossenbacher *et al.,* 2017). SI is absent or at best imperfectly

developed in gymnosperms (Zavada & Taylor, 1986 Runions & Owens, 1998; Kormatuk, 1999; Igic *et al*., 2008), so the high frequency of dioecy in gymnosperms may be a mechanism to avoid self-fertilization (Lloyd, 1974; Charlesworth & Charlesworth, 1978; Grossenbacher *et al*., 2017).

Mixed sexual systems (monoecy together with dioecy) are relatively rare in gymnosperms (14 species, *c*. 1%)*.* However, the extinct order Bennettitales had species with bisexual cones (hermaphroditic strobili) as well as species with unisexual cones (Owens *et al*., Friis, Pedersen, & Crane, 2009). *Pinus cembroides* var. *bicolor* is an interesting extant taxon, sometimes identified as a distinct species (*P. johannis* M.-F.Robert). The populations of this taxon are almost exclusively dioecious, making it the only completely dioecious taxon in the Pinaceae (Flores-Rentería *et al.*, 2013). Dioecy is also found, albeit rarely, in *Pinus edulis* (Floyd, 1983). Similarly, both dioecious and monoecious species can be found in the Podocarpaceae and Cupressaceae, often within the same genus, and indeed have species that can use both sexual systems. In comparison, in the Taxaceae family, *Taxus canadensis* Marshall is a completely monoecious species and monoecy is relatively common in *T. brevifolia* (DiFazio *et al.*, 1996). All these variations occur in a group with barely a thousand species, whereas angiosperms have about three hundred thousand (Christenhusz & Byng, 2016). High rates of extinction and niche conservatism could be the reason for the relatively small diversity of gymnosperms (Crisp & Cook, 2011). The largest family (Pinaceae) is made up of monoecious species, and many of them are found in cool climates, which affects our view of gymnosperms, the greatest diversity of which is found in the tropics.

This study shows that gymnosperms are an interesting model of sexual system evolution because of the relatively small number of monophyletic extant species (about 1000) . However, this model may have limitations since the extant taxa are distantly related which can

significantly influence any analysis of the gymnosperms. Our analyses have provided an important snap-shot of the current position of dioecy but do not take into account phylogenetic relationships, which would require a different analysis (Donoghue, 1989; Pagel & Harvey, 1988). However, any such analysis will require the data and insights provided here.

Acknowledgements

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Table 1. Sum of monoecious, dioecious and mixed (monoecious and dioecious) species in different gymnosperm families. The number of taxa was determined from Farjon (2010), Osborne *et al.* (2012) and Ickert-Bond and Renner (2016).

Family	Total species	Monoecious	Mixed	Dioecious	% dioecious
Araucariaceae[a]	37	35	0	2	5.4
Cupressaceae[a]	135	87	8	40	29.6
Cycadaceae[b]	107	0	0	107	100.0
Ephedraceae[c]	54	0	0	54	100.0
Ginkgoaceae[e]	1	0	0	1	100.0
Gnetaceae[c]	39	0	0	39	100.0
Pinaceae[a]	224	222	2	0	0.0
Podocarpaceae[ad]	178	6	3	169	94.9
Sciadopityaceae[a]	1	1	0	0	0.0
Taxaceae[a]	32	1	1	30	93.7
Welwitschiaceae[c]	1	0	0	1	100.0
Zamiaceae[b]	224	0	0	224	100.0
Total	**1033**	**352**	**14**	**667**	**64.6**

[a] Farjon (2010)

[b] Osborne *et al.* (2012)

[c] Ickert-Bond & Renner (2016)

[d] With the addition of one new species, *Podocarpus orarius* R.R.Mill & M.Whiting

[e] Page (1990)

Table 2. Grouping of gymnosperms according to growth forms and sexual dimorphism. The division into growth forms was based on the World Checklist of Selected Plant Families database from Raunkiaer (1934) with modifications made by De Langhe *et al.* (1983). As one species can occur in the form of various growth forms, the sum of species was given at the bottom of the table. Growth forms: phanerophytes (over 3 m high), nanophanerophytes (grows clearly above the ground but below 3 m), chamaephytes (grows closely to the ground level, always below 0.5 m) and climbing plants

Growth form	Total species	Monoecious	Mixed	Dioecious	% Dioecious
Phanerophytes	541	324	11	206	38.1
Phan./nano.	88	17	1	70	79.5
Nanophanerophytes	162	7	1	154	95.1
Nano./cham.	41	1	1	39	95.1
Chamaephytes	165	3	0	162	98.2
Climbing	36	0	0	36	100.0
TOTAL	1033	351	12	670	64.9
Σ Phanerophytes	629	341	12	276	43.9
Σ Nanophanerophytes	291	25	3	263	90.4
Σ Chamaephytes	206	4	1	201	97.6
Σ Climbing	36	0	0	36	100.0

Table 3. Occurrence of gymnosperms in particular climatic zones. The range of some species covers significant areas in more than one zone. Because one species can occur in many climate zones, the sum of species that occur in tropical, temperate, dry and cold areas is given at the bottom of the table. The Köppen-Geiger classification was used, which divides climates into five main climate groups: tropical, dry, temperate, cold and polar (Peel, Finlayson & McMahon, 2007)

Climate	Total species	Monoecious	Mixed	Dioecious	% Dioecious
Tropical	403	56	3	344	85.4
Trop./temp.	70	16	0	54	77.1
Trop./temp./dry	15	7	1	7	46.7
Temperate	324	134	3	187	57.7
Temp./dry	57	20	2	35	61.4
Temp./cold	54	45	2	7	13.0
Temp./cold/dry	12	11	0	1	8.3
Cold	39	36	1	2	5.1
Cold/dry	30	19	0	11	36.7
Dry	29	8	2	19	65.5
TOTAL	1033	352	14	667	64.6
Σ Tropical	488	79	4	405	83.0
Σ Temperate	532	233	8	291	54.7
Σ Dry	143	65	5	73	51.0
Σ Cold	135	111	3	21	15.6

Table 4. Dioecious, monoecious and mixed (monoecious and dioecious) gymnosperm species divided by pollination and cone type.

Pollen dispersal	Ambophily		Anemophily	
Seed dispersal	Zoochory	Anemochory	Zoochory	Anemochory
Dioecious	372	1	272	22
Mixed	0	0	12	2
Monoecious	0	0	16	336
Total	372	1	300	360

Table 5. Dioecious, monoecious and mixed (monoecious and dioecious) gymnosperm species divided according to the IUCN Red List of Threatened Species: LC - Least Concern, NT - Near Threatened, VU - Vulnerable, EN - Endangered, CR - Critically Endangered, EW - Extinct in the Wild. N – number of species. Dioecious and monoecious species are unequally distributed across these IUCN categories, Pearson's chi-squared test, p<0.0001

	Dioecious		Mixed		Monoecious		Sum	
	N	%	N	%	N	%	N	%
LC	222	33.4	11	78.6	179	50.7	412	39.9
NT	107	16.0	1	7.1	57	16.2	165	16.0
VU	109	16.3	0	0.0	47	13.4	156	15.1
EN	111	16.7	2	14.3	50	14.2	163	15. 8
CR	66	9.8	0	0.0	15	4.3	81	7.8
EW	4	0.6	0	0.0	0	0.0	4	0.4
Total	619	92.8	14	100	348	98.9	981	95.0

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Table 6. Dioecious and monoecious gymnosperm species divided according to the IUCN Red List of Threatened Species on two categories: LC - Least Concern, and TE – Threatened and Endangered (combined categories: NT - Near Threatened, VU - Vulnerable, EN - Endangered, CR - Critically Endangered, EW - Extinct in the Wild) in particular climatic zones. Fisher's exact test on 2 (dioecious and monoecious) x 2 (LC and TE) contingency table made separately in cold, dry, temperate and tropical climate. The number of species appears larger than in the database (see Appendix A) because species assigned to more than one climatic zone in the database are used in the analysis of every climate zone in which they occur.

Climate	Dioecious (N species)		Monoecious (N species)		P
IUCN	LC	TE	LC	TE	
Σ cold	17	4	83	28	0.7817
Σ dry	58	15	46	18	0.3231
Σ temperate	116	175	120	112	**0.0079**
Σ tropical	103	302	28	51	0.0728