Higher seed number compensates for lower fruit set in deceptive orchids

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Summary

1. Floral deception is widespread in orchids, with more than one-third of the species being pollinated this way. The evolutionary success of deceptive orchids is puzzling, as species employing this strategy are thought to have low reproductive success (less flowers yielding fruits) because of low pollination rates. However, direct measurements of total seed production in orchids – which is a better measure of reproductive success – are scarce due to the extremely small size of their seeds.

2. Here, we quantified seed numbers in 1015 fruits belonging to 48 orchid species from the Pannonian ecoregion (central Europe) and obtained fruit set and thousand-seed weight data for these species from the literature. We used phylogenetic comparative methods to test the hypothesis that deceptive species should compensate for their lower fruit set by having either more flowers, larger seeds or more seeds in a fruit.

3. Similarly to previous studies, we found that deceptive orchids have substantially lower fruits-set than nectar-rewarding ones. Also, we found that deceptive species have more seeds in a fruit but not more flowers or larger seeds compared to nectar-rewarding ones. Based on our results, deceptive species compensate for their lower fruit set by having higher seed numbers per fruit. As a consequence, their seed numbers per shoot do not differ from that of nectar-rewarding ones.

4. Together with other benefits of deceptive pollination (e.g. lower energy expenditure due to the lack of nectar production and higher genetic variability due to decreased probability of geitonogamous pollination), our results can explain why deceptive strategies are so widespread in the orchid family.

5. *Synthesis.* Our results indicate that deceptive orchids can compensate for their lower fruit set by having more (but not larger) seeds in a fruit than rewarding species. These findings highlight possible ways in which plants can increase their reproductive success in face of pollinator limitation. We emphasize that fruit set in itself is an inappropriate measure of the reproductive success of orchids – the total number of seeds per shoot is a much better approximation.

Key-words: deception, nectar reward, Orchidaceae, phylogenetic comparative methods, pollination, reproductive ecology, reproductive success, seed mass, seed number, thousand-seed weight

Introduction

Reproduction in plants requires transfer of pollen from male to female parts, which is often achieved by enlisting animal pollinators, such as insects, bats or birds (Stpiczyńska 2003).

Most plants attract pollinators by supplying them with food, typically nectar and/or pollen. Some plants, however, do not provide any reward but rely on deception, for example by mimicking the flowers of nectar-rewarding species or the females of insect pollinators. Floral deception evolved independently in at least 32 angiosperm plant families (Renner 2006) and is especially common in orchids, where more than one-third of the species (>6500 species) is pollinated

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deceptively (Girord *et al.* 2002). Within orchids, deceptive pollination evolved multiple times independently, which might have played a key role in their extraordinary diversification (Papadopulos *et al.* 2013). Possible reasons for the disproportionate frequency of deception in the orchid family compared to other angiosperm families have been assessed by Jersáková, Johnson & Kindlmann (2006).

The most common floral deception among orchids is food deception, where flowers resemble nectar-producing flowers (brightly coloured floral parts, sweet smelling fragrances), without producing any nectar. Sexual deception, where flowers mimic the chemical signals, visual appearance and tactile perception of female insects (mostly attracting Hymenopterans), is also quite frequent. Deceptive orchids mostly rely on naive pollinators, since insects quickly learn to identify non-rewarding flowers (Ferdy *et al.* 1998). Thus, pollination success in deceptive orchids is substantially lower than in nectar-rewarding ones. According to Neiland & Wilcock (1998), the mean fruit-set (the proportion of flowers that develop into fruits) of non-rewarding species in Europe is 27.7%, which is significantly lower than the mean fruit set of rewarding species, 63.1%. In agreement with the former results, Tremblay *et al.* (2005) also found a significant difference between the mean fruit set of rewarding and non-rewarding species considering both temperate and tropical species (37.1% and 20.7%, respectively).

The evolutionary success of deceptive orchids is surprising in the light of their reduced reproductive success and is generally explained by two, mutually non-exclusive hypotheses (Jersáková, Johnson & Kindlmann 2006). First, instead of nectar production (which is generally resource demanding, Southwick 1984), the plant might allocate resources directly to reproduction, that is to fruit and seed production. Secondly, nectarless flowers decrease the chance of pollinator-mediated geitonogamy, as pollinators visit fewer flowers on the same plant and outcrossing by xenogamy will be promoted (Johnson & Nilsson 1999; Johnson, Peter & Ågren 2004; Jersáková, Johnson & Kindlmann 2006). The phenomenon of rewarding plants suffering from reduced offspring fitness due to self-pollination is known in other angiosperm plants as well (e.g. de Jong, Waser & Klinkhamer 1993).

The above inference is based on the assumption that low pollination success results in reduced reproductive success, since fruit set is generally used as the sole measure of reproductive success in orchids (Neiland & Wilcock 1998; Kull 2002). However, low pollination success might be compensated by at least three mechanisms: producing (i) more flowers, (ii) larger seeds or (iii) more seeds per fruit. This 'compensation hypothesis' has not been tested to date, partly because of methodological reasons: orchids have extremely light dust seeds produced in very high numbers (van der Pijl 1982; Arditti & Ghani 2000), and the unusually high number of minute and low-weight seeds causes difficulties in the estimation of seed production (Proctor & Harder 1994; Nazarov 1998). Due to these methodological limitations, very few data have been published on the seed production of orchids. Seed number data of only 17 European species were published by Arditti & Ghani (2000).

Here, we test the 'compensation hypothesis', which predicts that, if compensation for reduced fruit set occurs in deceptive orchids, these species should have more flowers and/or larger seeds and/or more seeds per fruit than nectar-rewarding ones. Although these are not mutually exclusive, compensating by having both bigger seeds and more seeds does not seem likely, as a trade-off between the size and number of seeds is presumable (e.g. Mazer 1987; Gundel *et al.* 2012). This trade-off can work at the fruit and at the individual level as well. At the fruit level, limited space may be the most important, but at the individual level, resource limitation may be the key factor (Ackerman & Montalvo 1990; Mattila & Kuitunen 2000). To test the 'compensation hypothesis', we compared species with different pollination types in terms of flower number, seed size (thousand-seed weight) and capsular seed number (number of seeds per fruit) using phylogenetic comparative methods.

Materials and methods

DATA COLLECTION

We quantified seed numbers in a total of 1015 fruits of 48 orchid taxa, which is nearly three times the number of European species for which seed number data were available to date (Arditti & Ghani 2000). Field sampling took place during 2009 and 2010 in several locations across the Pannonian ecoregion (central Europe), during which fruits of 47 orchid species were collected. We also collected mature, but intact fruits from herbarium specimens in the herbarium of the Department of Botany, University of Debrecen (DE), which resulted in fruit samples for 20 orchid species. In total, we collected 22.9 ± 3.9 (mean ± SE) fruits from 3.6 ± 0.4 (mean ± SE) different locations per species. Undehisced fruits were usually collected 4–6 weeks after flowering.

Harvested fruits were stored in open Eppendorf tubes or scintillation vials depending on their size. Fruits were left to dry on room temperature and were squashed by a metal needle so that in every Eppendorf tube all seeds of the fruit and small parts of the pericarp could be found. A known volume of glycerine (99.5%) was then pipetted into each tube and the content was stirred by hand, using a metal needle (homogenization using a shaker proved to be unsatisfactory). In the highly viscous glycerine, the very low-density seeds rose to the surface more slowly (several minutes) than in water (almost immediately). This allowed us to make a suspension of seeds and then count the number of seeds in drops of glycerine as follows. 10×4 µL of the freshly stirred samples was pipetted onto object slides, and the number of seeds in every drop was counted under a light microscope. The tip of the automatic pipette tips (1–10 µL) was cut at an angle of cc. 45 degree in order to enable orchid seeds to be imbibed. The number of seeds in a fruit was assessed based on the counted seed numbers in the drops and the proportion of the drops to the whole volume.

To investigate whether the well-known difference between the fruit set of deceptive and nectar-rewarding species holds true for the studied species, we used fruit set data provided by Molnár V. (2011) mostly from Hungary, and in some cases (species for which Hungarian data was not available) data from several European countries published by Claessens & Kleynen (2011) (Table 1). To study whether deceptive species have larger seed sizes, thousand-seed weight data (measured with an accuracy of 0.0001 g) were obtained from the data

Table 1. Characteristics of the 48 species studied

Taxon	FS	FLN	TSW (g)	CSN	n	L	FRN	SNS	Poll	Hab	GH	nrITS source (GenBank acc. no.)
Anacamptis coriophora	88.2	18.2	0.0024	3192 ± 340	26	8	16.1	51257	NR	O	T	AY369086
Anacamptis morio	49.6	9.3	0.0010	4978 ± 521	20	7	4.6	22926	D	O	T	AY364881
Anacamptis palustris agg.	77.1	11.7	0.0019	7379 ± 535	38	1	9.0	66410	D	O	T	KP742370‡
Anacamptis pyramidalis	46.3	41.6	0.0016	2262 ± 205	23	3	19.1	43282	D	O	T	AY364870
Cephalanthera damasonium	75.5	5.5	0.0028	4528 ± 677	27	11	4.1	18631	A	F	R	AY146446
Cephalanthera longifolia	16.8	11.3	0.0040	4231 ± 342	21	3	1.8	7687	D	F	R	AY146447
Cephalanthera rubra	13.0	7.2	n.d.	4725 ± 840	12	4	0.9	4379	D	F	R	AY369084
Dactylorhiza fuchsii	63.5	26.6	0.0018	5205 ± 914	27	2	16.9	87786	D	O	T	DQ022864
Dactylorhiza incarnata agg.	75.2	33.6	0.0025	7076 ± 881	27	8	25.3	178710	D	O	T	DQ022885
Dactylorhiza majalis	54.6	20.0	0.0021	9639 ± 421	62	1	10.9	105229	D	O	T	DQ074217
Dactylorhiza sambucina	48.1	11.5	n.d.	3014 ± 168	85	2	5.5	16652	D	O	T	DQ074239
Dactylorhiza viridis	60.0	17.4	0.0022	1453 ± 136	11	4	10.4	15157	NR	O	T	DQ022880
Epipactis albensis	78.3	13.5	0.0030†	5997 ± 553	6	1	10.6	63570	A	F	R	KP204478‡
Epipactis atrorubens	70.4	21.9	0.0046	3226 ± 618	7	4	14.4	46596	NR	F	R	FR750398
Epipactis bugacensis	77.8	11.9	0.0034	4142 ± 1067	8	1	9.2	38188	A	F	R	KP204479‡
Epipactis helleborine	73.9	20.9	0.0024	6486 ± 677	7	2	8.7	56518	NR	F	R	EF153104
Epipactis leptochila	82.0	12.2	n.d.	3154 ± 280	9	1	10.0	31544	A	F	R	KP204480‡
Epipactis mecsekensis	76.8	9.7	0.0022	4266 ± 391	4	1	7.4	31705	A	F	R	KP204481‡
Epipactis microphylla	75.0	8.1	0.0035	2729 ± 379	8	3	6.1	16574	A	F	R	FR750399
Epipactis neglecta	81.6	13.8	0.0021	4761 ± 901	8	2	11.2	53470	A	F	R	KP204482‡
Epipactis nordeniorum	84.3	10.9	0.0016	4636 ± 669	8	2	9.2	42618	A	F	R	KP204483‡
Epipactis palustris	62.7	15.4	0.0030*	5750 ± 690	11	6	9.6	55486	NR	O	R	AY146448
Epipactis pontica	67.9	10.9	0.0019	2651 ± 309	6	3	5.8	15243	A	F	R	KP204484‡
Epipactis tallosii	75.4	16.8	0.0019	4121 ± 354	13	4	10.8	44459	A	F	R	KP204485‡
Epipactis voethii	n.d.	7.9	0.0025	6102 ± 1189	17	3	n.d.	n.d.	A	F	R	FR750400
Goodyera repens	70.1	12.6	0.0017	2509 ± 224	10	1	8.8	22123	NR	F	R	HM021556
Gymnadenia conopsea	73.4	36.9	0.0031	5005 ± 782	34	12	27.5	137851	NR	O	T	DQ351281
Gymnadenia odoratissima	86.4	47.2	0.0017	1278 ± 176	26	2	41.4	52924	NR	O	T	KT318278‡
Himantoglossum adriaticum	44.1	28.9	0.0013	10686 ± 1550	22	3	12.7	136145	D	O	T	FR750401
Himantoglossum jankae	30.8	30.6	0.0006	12085 ± 1124	29	3	9.4	113717	D	O	T	FR750402
Limodorum abortivum	68.0	12.4	0.0034	3623 ± 808	9	4	8.3	30034	A	O	R	AY351378
Liparis loeselii	73.0	12.7	n.d.	11354 ± 587	14	2	9.2	105023	A	O	T	AJ551453
Neotinea tridentata	43.3	23.2	0.0015	6064 ± 361	69	4	10.0	60806	D	O	T	Z94113-4
Neotinea ustulata	42.8	48.7	0.0010	2787 ± 519	11	3	20.8	58022	D	O	T	FR750397
Neottia nidus-avis	85.6	35.8	0.0031	2774 ± 303	24	4	30.6	84983	A	F	R	AY351383
Neottia ovata	94.8	34.9	n.d.	910 ± 211	4	2	33.1	30104	A	F	R	FJ694841
Ophrys apifera	84.4	4.0	0.0011	8068 ± 912	15	3	3.3	26949	A	O	T	AM980999
Ophrys fuciflora	67.4	5.1	0.0020*	5165 ± 835	3	3	3.4	17769	D	O	T	AJ972932
Ophrys oestrifera	24.8	4.4	0.0017	19726 ± 4189	8	2	1.9	37874	D	O	T	AM981015
Ophrys sphegodes	36.7	4.5	n.d.	16041 ± 2097	28	9	1.6	26308	D	O	T	AJ973255
Orchis militaris	38.1	28.4	0.0014	10948 ± 3274	9	4	10.8	118243	D	O	T	AY699977
Orchis pallens	38.3	16.5	0.0023*	6139 ± 1393	8	1	6.3	38678	D	F	T	KT318277‡
Orchis purpurea	26.8	39.0	0.0016	7360 ± 580	13	5	10.5	76983	D	O	T	AY364882
Orchis simia	45.1	20.3	0.0007	5780 ± 355	34	2	9.2	52941	D	O	T	Z94107-8
Platanthera bifolia	71.5	17.5	0.0013	6146 ± 325	99	12	12.6	77261	NR	F	T	KT962125‡
Platanthera chlorantha	49.7	14.1	0.0010	5295 ± 650	11	1	7.0	37280	NR	F	T	Z94117-8
Spiranthes spiralis	66.5	18.1	n.d.	3527 ± 383	6	2	12.0	42320	NR	O	T	FJ473354
Traunsteinera globosa	60.8	53.2	n.d.	2486 ± 226	38	2	32.4	80426	D	O	T	KT318279‡

Codes: FS – fruit set (%, as published by Molnár V. (2011) and Claessens & Kleynen (2011); FLN – average number of flowers; TSW (g) – thousand-seed weight (in grams) – based on the data set of Török *et al.* (2013) CSN – capsular seed number (mean ± SE); *n* – number of fruits studied; L – number of collecting localities; FRN – average number of fruits according to Molnár V. (2011); SNS – seed number per shoot; Poll – pollination type: A – autogamous, D – deceptive, NR – nectar-rewarding; Hab – habitat preference: O – open habitats, F – forest habitats; GH – growth habit: T – tuberous, R – rhizomatous.

*Measurements carried out for this study, following the methodology of Török *et al.* (2013).

†Data from Ljubka *et al.* (2014).

‡nrITS sequence generated for this study.

base of Török *et al.* (2013). Seed numbers per shoot were obtained by multiplying the mean capsular seed number of each species by the fruit number of the given species. Fruit and flower numbers are the means of multiple measurements done by Molnár V. (2011). (Note that flower number, fruit set and fruit number data used here were measured not just on the individuals from which seed numbers originated, but on several other individuals too; thus, these data are based on a larger number of observations.)

To study the effect of pollination type on fruit set, flower number, thousand-seed weight, capsular seed number and seed number per shoot, taxa were categorized following Claessens & Kleynen (2011) (Table 1). We distinguished nectar-rewarding, deceptive (food-deceptive and sexually deceptive) and autogamous groups. Both facultative and obligate autogamy were considered as autogamy (self-pollination), as these strategies both can be considered to be independent from pollinators (Molnár V. *et al.* 2012). We controlled for habitat preference and growth habit, for which we distinguished species of open habitats and species of shaded habitats, and rhizomatous and tuberous species according to Kull & Hutchings (2006).

PHYLOGENETIC TREE RECONSTRUCTION

In order to provide a phylogenetic framework for the studied species, we used sequences of the nuclear ribosomal internal transcribed spacer (nrITS), one of the most heavily used phylogenetic markers in species-level systematic studies in plants (Baldwin *et al.* 1995; Álvarez & Wendel 2003; Nieto-Feliner & Rosselló 2007). The sequences were obtained – if available – from GenBank, otherwise we generated the sequences newly. In this latter case, we used field-collected leaf samples dried in silica gel and followed a modified CTAB protocol to isolate total genomic DNA. The details of the laboratory procedures for DNA extraction and the amplification of the nrITS region are given in more details in, for example, Sramkó *et al.* (2014). The successfully amplified samples were sent to Macrogen Inc. (Seoul, South Korea) for Sanger sequencing from the forward and reverse directions. The sequences obtained were checked for intra-individual polymorphism (see Nieto-Feliner & Rosselló 2007); if an additive polymorphic site was detected, it was coded with IUPAC ambiguity nucleotide codes (Cornish-Bowden 1985). All newly generated sequences were uploaded to GenBank (for accession numbers, see Table 1).

The nrITS region of our samples was aligned manually in BioEdit v.7.1.3 (Hall 1999); then, the aligned matrix of 686 nucleotide length was used to reconstruct the phylogenetic relationships of our studied species under the maximum parsimony (MP) criterion in PAUP* v4.0b10 (Swofford 2003). Given the geographically limited sampling (orchids of central Europe) for this study, there were evidently large gaps in our taxonomic sample coverage for the phylogenetic work. Therefore, we used the well-established molecular system of European orchids (Bateman *et al.* 2003, 2005; Bateman 2009) as a backbone constraint (see Fig. 1) to fix the relationship between the main lineages of the European orchids studied; and to avoid the potential drawback of a suboptimal taxonomic sampling. Phylogenetic trees compatible with the above-mentioned constraint were searched in a heuristic way under the MP criterion in PAUP* applying all the default settings but holding 10 trees in each iteration step and running 1000 random stepwise additions. The root of the tree was specified by assigning the species of the Epidendroideae subfamily as outgroup (Cameron *et al.* 1999; Górniak, Paun & Chase 2010; Chase *et al.* 2015). To assess the robustness of our tree, we ran the nonparametric bootstrap test (Felsenstein 1985) as implemented in PAUP* using 1000 pseudo-replications. Finally, one of the most parsimonious trees with branch lengths was transformed to an ultrametric tree by the nonparametric rate smoothing algorithm (Sanderson 1997) as
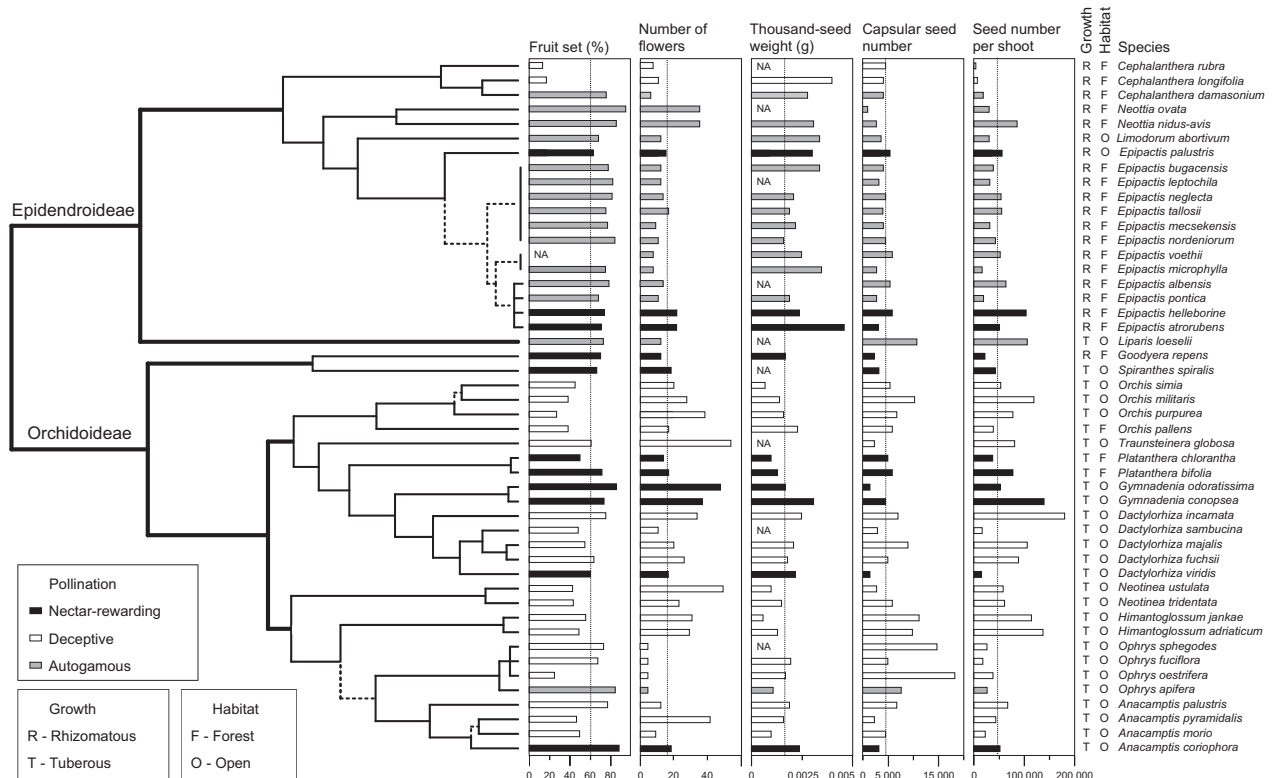
Fig. 1. The favoured phylogenetic tree of 28 equally most parsimonious trees made ultrametric by nonparametric rate smoothing and used in all subsequent analyses as phylogenetic control. The backbone constraint applied in the heuristic MP search is indicated by thick branches, whereas branches receiving low statistical support (50–75%) in our bootstrap analysis are dashed. Trait values for fruit set, flower number, thousand-seed weight, capsular seed number, seed number per shoot, growth habit and habitat preference for each taxon are given next to the tree. Dashed vertical lines indicate median values for each trait.

implemented in r8s v.1.71 (Sanderson 2003). This procedure allowed us to generate branch lengths proportional to genetic distance between the species, and the resulting ultrametric tree (Fig. 1) was used as input for analyses using the phylogenetic control.

The heuristic search with 1000 random replicates found in 997 instances the same 28 equally most parsimonious trees. Between the trees found, the position of the micro-species of *Epipactis helleborine sensu lato* and some notoriously problematic species pairs (e.g. *Orchis militaris* and *O. purpurea*) was incongruent. These branches received no (<50%) or low (<75%) statistical support in the nonparametric bootstrap procedure (Fig. 1); otherwise, our trees are compatible with the established phylogenetic relationship of European orchids (Bateman *et al.* 2003, 2005; Bateman 2009). Thus, we selected one of the 28 trees (see Fig. 1) to represent the phylogenetic relatedness between our samples, and this was made ultrametric for the subsequent analyses applying phylogenetic control.

COMPARATIVE ANALYSES

To study the relationship between traits while controlling for phylogenetic relatedness, we used Bayesian phylogenetic mixed models (BPMMs), as implemented in the MCMCglmm package (Hadfield 2010), in the R Statistical Environment (R Core Team 2013). BPMMs are similar to traditional linear mixed models with the important difference that they can incorporate hierarchical random effects arising, for example, from pedigrees or phylogenetic trees. In this way, the non-independence of data points arising from shared phylogenetic descent of taxa can be taken into account when evaluating the relationship between traits.

Seed production of plants depends on a number of biotic and abiotic factors in general (e.g. Leishman 2001; Moles *et al.* 2005a,b; Gundel *et al.* 2012), but little is known about the role of these factors in shaping interspecific variation in seed size/number in orchids. To take into account potential confounding factors, we controlled for habitat shadiness (open or shady) which has been shown to affect seed production (e.g. Nakagoshi 1985; Csontos 1998; Milberg, Andersson & Thompson 2000; Fenner & Thompson 2005). We also controlled for growth habit (tuberous or rhizomatous), as it may affect the amount of reserves stored in below-ground organs, which can also affect reproduction. To test our hypothesis, we built a full model that contained pollination type and potential confounding factors, that is habitat preference and growth habit. We also used a reduced model which contained only pollination type. We applied both models to analyse fruit set, flower number, thousand-seed weight, capsular seed number and seed number per shoot using the species-level data set (i.e. data points were individual taxa, as seen in Table 1). (Note that analysing capsular seed number on the individual level resulted in essentially identical results; see S1 in Supporting Information). Capsular seed number and seed number per shoot were log-transformed to obtain a normal distribution, and BPMMs with Gaussian error structure were employed. Fruit set (which was a proportion variable) was analysed using binomial BPMM with the number of fruits (successes) and the number of unfertilized flowers (failures) as a bivariate response. To account for overdispersion, an observation-level random effect was added to this model (Harrison 2014).

All models were run for 550 000 MCMC iterations, using a burn-in of 5000 iterations and a thinning interval of 500 iterations. We used parameter-expanded priors for the random effects (MCMCglmm code: $V = 1$, $v = 0.002$). All models were run multiple times ($N > 3$) and MCMC chains were visually checked to ensure that convergence was achieved.
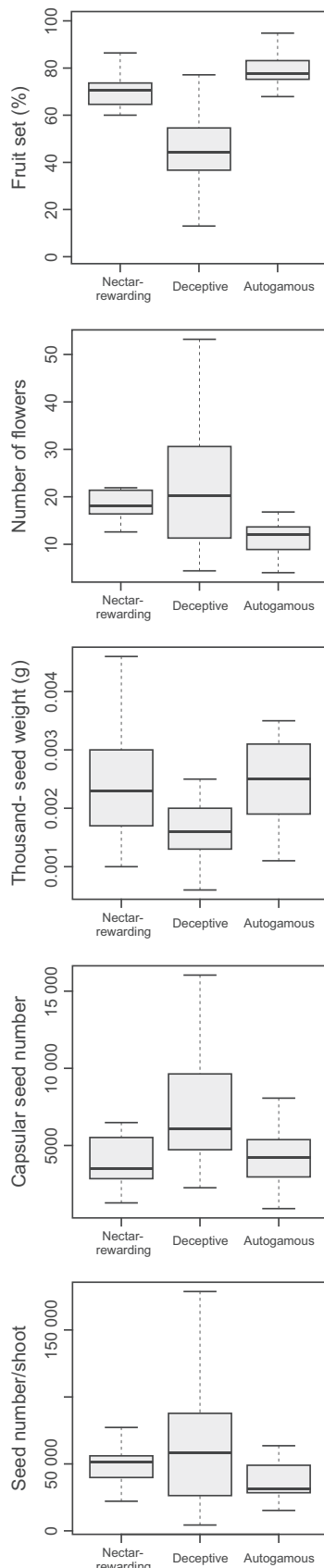
Results

Capsular seed numbers ranged from 910 to a maximum of 19,726 (Table 1). On average, deceptive species set about two times as many seeds in a capsule as nectar-rewarding ones (Fig. 2). We calculated seed numbers per shoot for 47 orchid species (for which average fruit numbers were available). Seed numbers per shoot ranged from 4379 to 178 710 (Table 1). The highest seed number per shoot was recorded for deceptive species, but the difference between the pollination types was not as pronounced as in the case of capsular seed numbers (Fig. 2).

Pollination type had a significant effect on fruit set: the fruit set of deceptive species was found to be significantly lower than that of nectar-rewarding ones (Table 2). Pollination type had no effect on thousand-seed weight, but significantly affected capsular seed number, as capsular seed numbers of deceptive species were found to be significantly higher than that of nectar-rewarding ones (Table 2). Flower number was affected by pollination type as autogamous species had significantly fewer flowers than nectar-rewarding ones, but there was no difference between the flower number of nectar-rewarding and deceptive species. We also analysed the effect of pollination type on seed number per shoot, but we found no difference between the seed number per shoot of different pollination types. Autogamous species differed significantly from nectar-rewarding ones only regarding the number of flowers (see Table 2 and Fig. 2). Habitat preference and growth habit had no effect on any of the studied variables (see Table S2).

Discussion

Consistent with previous studies (Neiland & Wilcock 1998; Tremblay *et al.* 2005; Molnár V. *et al.* 2015), we found that deceptive orchids have significantly lower fruit set than nectar-rewarding taxa. This lower fruit set is often seen as a sign of reduced fitness (Neiland & Wilcock 1998; Tremblay *et al.* 2005) resulting from the ability of pollinators to avoid non-rewarding flowers (Ferdy *et al.* 1998). However, our findings suggest that this is not necessarily true – low fruit set does not equal low reproductive success, since deceptive taxa can effectively compensate for their reduced pollination success by producing more seeds per fruit. As a result, total seed numbers per shoot recorded in this study did not differ between pollination types.

Compensation for reduced pollination success has been suggested previously to occur in tropical orchids (Neiland & Wilcock 1998). In their study, Neiland & Wilcock (1998) showed that tropical species are only about one-third as successful as temperate ones based on their average fruit set values (13.6% and 38.2%, respectively). Similarly to our hypothesis, they suggested that tropical orchids may compensate for their very low fruit set by having more seeds in a fruit, as they found that the nine tropical species for which Arditti & Ghani (2000) provided seed number data have about 150 times more seeds in a fruit than the eight temperate

Fig. 2. Fruit set (%), flower number, thousand-seed weight, capsular seed number and seed number per shoot of orchids with different pollination types.

Table 2. Results of BPMMs on the effect of pollination type, habitat preference and growth habit on the fruit set, flower number, thousand-seed weight, capsular seed number and seed number per shoot of species

	Posterior mean	*P*-value
Fruit set		
(Intercept)	1.036	**0.004**
Pollination – Deceptive	−1.436	**<9e-04**
Pollination – Autogamous	0.321	0.477
Number of flowers		
(Intercept)	21.745	0.095
Pollination – Deceptive	−1.037	0.805
Pollination – Autogamous	−7.230	**0.037**
Thousand-seed weight		
(Intercept)	0.002	0.780
Pollination – Deceptive	−0.000	0.939
Pollination – Autogamous	−0.001	0.903
Capsular seed number		
(Intercept)	12.293	**<9e-04**
Pollination – Deceptive	1.057	**0.038**
Pollination – Autogamous	0.175	0.745
Seed number per shoot		
(Intercept)	10.694	**<9e-04**
Pollination – Deceptive	0.026	0.917
Pollination – Autogamous	−0.205	0.602

Significant effects are marked with boldface.

species. Although this result is based on a relatively small number of observations, the parallelism between this study and our results is rather obvious. Hence, this type of compensation might be a general strategy in orchids experiencing low pollination success and reduced fruit set.

Producing more flowers could effectively compensate for the low fruit set of deceptive species, but it could be energetically wasteful to produce a large number of flowers that do not have good chances to be pollinated. Instead of producing more flowers, deceptive species compensate by producing a larger number of seeds in fruits. Larger seed size could also compensate for lower fruit set; as in the case of other plant species, it seems to positively affect germination (e.g. Eriksson 1999; Jakobsson & Eriksson 2000), seedling establishment and performance (e.g. Moles *et al.* 2004; Baroloto, Forget & Goldberg 2005) and the subsequent growth and reproductive capacity of the plant (e.g. Stanton 1985; Tremayne & Richards 2000). However, we did not find any difference between thousand-seed weights of pollination types; thus, deceptive species do not seem to compensate for their lower fruit set by having larger seeds.

Although deceptive orchids can compensate in seed numbers, having more seeds in a fruit might have disadvantages as well. For instance, it might be associated with strongly clustered seed distribution after dispersal, resulting in high rates of intraspecific seedling competition (Cheplick 1992) and lowered rates of survival to adulthood. As a seed size–seed number trade-off probably works at the fruit level too (e.g. Ågren 1989; Méndez 1997; Eriksson 1999), higher capsular seed numbers may also result in smaller seeds or seeds with less resources, although our results do not seem to

support this assumption. Moreover, when roughly the same number of seeds per shoot is packed in a smaller number of fruits, losing one of the fruits (e.g. due to herbivory) can result in a higher amount of loss considering the whole amount of seeds. Further factors can also be involved in reproductive success (i.e. germination potential or seedling establishment), and these might differ among pollination types, but the evaluation of these factors is far beyond the scope of the present study. The estimation of viability in orchid seeds is extremely difficult and time-consuming even by chemical staining or in vitro germination (e.g. Vujanovic *et al.* 2000; Hosomi *et al.* 2012; Lemay *et al.* 2015), and there is little information on how in vitro and in vivo processes relate to each other (but see e.g. Rasmussen & Whigham 1993). Similarly, there is not enough information available about in situ seedling establishment and survival to draw general conclusions (but see e.g. Batty *et al.* 2001, 2006; Scade *et al.* 2006). Because of these factors, the relatively high number of seeds produced by deceptive orchids might not fully compensate for their low pollination success – future studies are required to test the above assumptions.

There are known benefits of deceptive pollination, such as (i) lower energy expenditure due to the lack of nectar production and (ii) higher genetic variability due to decreased probability of geitonogamous pollination (Jersáková, Johnson & Kindlmann 2006). Jersáková, Johnson & Kindlmann (2006) also admitted that despite these benefits, the evolutionary stability of this strategy is hard to explain, as, at least when pollinators are scarce, mutations for nectar production would easily spread through the whole population. The benefits mentioned above together with the seed production patterns we found in the present study can at least partly explain why deceptive strategies are so widespread in the orchid family. It also points out that multiple aspects of reproductive output need to be taken into account to fully evaluate the reproductive success of an evolutionary strategy.

Based on our results, we can state that fruit set in itself is not sufficient to evaluate the reproductive success of orchids, and reproductive success of deceptive species is not necessarily lower than that of nectar-rewarding ones. This can explain results like that of Jacquemyn *et al.* (2005), who have found that, despite their higher fruit set, nectar-rewarding orchids are not less threatened by local extinction and distribution decline than deceptive ones. Our results indicate that seed number per shoot can be the best and easiest approximation of orchid reproductive success to date, and also draw the attention to the need of research on the above-mentioned poorly known topics and their relationship with the fruit and seed production patterns observed by us.

This study also raises several interesting questions about plant adaptations to low pollination success. Is the difference in seed numbers per fruit an evolutionary adaptation or a result of phenotypic plasticity? If the latter is true, compensation could occur on the plant level as well (i.e. individual plants adjusting seed number per fruit to pollination success) – a prediction that could be easily tested in field experiments by manipulating access of pollinators to flowers. Another important question pertains to the origin and maintenance of these patterns of seed production. Many plant populations can be severely affected by the observed pollinator declines (Biesmeijer *et al.* 2006) and a diverging phenology of plants and their pollinators due to climate change (Kudo & Ida 2013). Phenological mismatch may effect deceptive orchids especially strongly (Molnár V. *et al.* 2012; Robbirt *et al.* 2014). This could mean that some of the differences between nectar-rewarding and deceptive orchids that we observe today could be a recent phenomenon caused by climate change or pollinator decline, although evidence for such a link is lacking to date (see Molnár V. *et al.* 2015). How these pollination systems will cope with the challenges of a changing world remains an important question.

Data accessibility

All seed number data and phylogenetic data used in the analyses are available from Dryad – http://dx.doi:10.5061/dryad.7hn84 (Sonkoly *et al.* 2015).

nrITS sequences generated for this study are available from GenBank, for accession numbers see Table 1.

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Supporting Information

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

Table S1. Results of BPMMs on the effect of pollination type, habitat preference and growth habit on capsular seed number on the individual level.

Table S2. Results of BPMMs on the effect of pollination type, habitat preference and growth habit on fruit set, flower number, thousand-seed weight, capsular seed number and seed number per shoot (full models).