

Reduced fecundity and genetic diversity in small populations of rewarding versus deceptive orchid species: a meta-analysis

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Background and aims – Understanding how plant traits mediate the relationship between plant population size, fitness and genetic diversity is fundamental to conservation. Many orchid species are threatened, possibly due to their susceptibility to the consequences of habitat fragmentation and the associated decreasing population size. One third of all orchids do not produce nectar to entice pollinators, and this is believed to promote gene flow and outcrossing. Therefore, we hypothesised that deceptive orchids are less susceptible to reductions in population size than rewarding species.

Methods – We reviewed the available literature and used traditional and phylogenetically independent meta-analyses to explore relationships between population size, fitness (fruit set) and genetic diversity.

Key results – Results showed that orchids in general are highly susceptible to the consequences of small population size. Fruit set significantly decreased in small populations of rewarding, but not in deceptive species. Deceptive orchids were, however, as likely to lose genetic diversity in small populations, even though significantly less genetic differentiation (F_{st}) was present between populations of deceptive orchids. Finally, we found that homozygosity did not relate with population size, which may indicate a selection process against less fit homozygotes in small orchid populations.

Conclusions – We conclude that reductions in population size most likely resulting from habitat loss and fragmentation represent major threats to the population viability of orchids. Fitness of rewarding orchids declines through ecological mechanisms, whereas deceptive orchids may be more susceptible to genetic loss as they are more susceptible to decreased gene flow.

Key words – Deceptive orchids, fruit set, genetic variation, genetic differentiation, habitat fragmentation, pollination system.

INTRODUCTION

The effects of small plant population size on fitness and genetic diversity are of fundamental importance in plant ecology, evolution and conservation. Decreasing habitat quality and habitat loss and fragmentation, all major drivers of the present biodiversity crisis (Young & Clarke 2000), have caused severe decreases in population size in numerous plant species. Since small plant populations are more prone to extinction (e.g. Schleuning et al. 2009, Dornier & Cheptou 2012), it is of major importance to understand how plant traits mediate the susceptibility of plant species to decreasing population size in terms of genetic diversity and reproduction.

Ecological mechanisms, such as pollinator and pollen limitation, reduce the fitness in small populations through reduced reproductive success (e.g. Ågren 1996). Additionally also genetic mechanisms, especially reduced genetic

variability is expected to be an important cause of decreasing viability in small populations (Young et al. 1996). Genetic variation can be lost through random genetic drift, i.e. random variation in allele frequencies between generations in finite populations (Honnay 2013). Furthermore, elevated inbreeding in small populations will increase the degree of homozygosity (Keller & Waller 2002). Inbreeding in plants can result from mating between related individuals, or from increased selfing, either through geitonogamy or autogamy (Honnay & Jacquemyn 2007, Aguilar et al. 2008, Angeloni et al. 2011). Loss of genetic variation through drift and increased homozygosity may have important consequences for the long and short term survival chances of a population. In the longer term, low genetic variability can constrain the evolutionary potential of plant populations (Booy et al. 2000), which may be especially problematic when environmental conditions are rapidly changing. In the short term, the expression of deleterious recessive alleles through in-

creased homozygosity may jeopardize reproductive success (Charlesworth & Willis 2009).

Orchidaceae is one of the most species-rich plant families in the world, estimated to contain more than 27,000 species (Chase 2005). Orchids, however, are also predicted to have the highest proportion of threatened or extinct species of all plant families (Swarts & Dixon 2009). This is likely the result of specific life history traits that typify most orchid species. In general, orchids are habitat specialists with special requirements regarding both abiotic and biotic environmental quality (Swarts & Dixon 2009). Most orchid species rely on biotic interactions with both specific mycorrhizal fungi (Smith & Read 2008, Rasmussen & Rasmussen 2009) and pollinators (van der Pijl & Dodson 1966, van der Cingel 1995) for completion of their life cycle. Even seemingly minor environmental changes can alter these interactions and elicit a strong negative impact on plant performance, leading to decreasing population sizes and eventually local extinction. It is therefore of conservation importance to understand the deleterious consequences of declining population sizes in orchids (Swarts & Dixon 2009), especially since their decline might be a first sign of a habitat degradation process.

Although orchids exhibit complex floral adaptations to pollinators, two broad categories of pollination systems can be discerned within the orchid family (Neiland & Wilcock 1998, Tremblay et al. 2005). Whereas most species offer substantial nectar rewards to entice pollinators (further referred to as 'rewarding orchids'), one third of the species mimics odours and visual stimuli to deceive pollinators with the promise of food or reproduction (further referred to as 'deceptive orchids') (Cozzolino & Widmer 2005). A prevailing hypothesis for the existence of pollination by deceit is the outcrossing hypothesis, stating that deceptive pollination promotes outcrossing (Dressler 1981, Nilsson 1992, Jersáková et al. 2006). In contrast to pollinators that receive a reward, deceived pollinators rapidly learn to avoid rewardless plants (i.e. pollinator learning) and are more likely to leave for a different population, increasing pollen flow and decreasing genetic differentiation among populations (Peakall & Beattie 1996, Johnson 2000, Jersáková et al. 2006). Furthermore, rewarded pollinators tend to visit more flowers on the same inflorescence, spend more time on the same flower and visit neighbouring conspecific individuals (Johnson et al. 2004, Jersáková et al. 2008). Although this behaviour generally increases the percentage fruit set in rewarding orchids as compared to deceptive species (Tremblay et al. 2005), it also results in higher geitonogamous pollination, and potentially in inbreeding depression in rewarding species (Smithson & Macnair 1997). For instance, Johnson et al. (2004) predicted that nectar production in the deceptive orchid *Anacamptis morio* (L.) Bateman, Pridgeon & M.W.Chase (syn. *Orchis morio* L.) would result in a 40% increase in geitonogamous pollination. On the other hand, Smithson (2006) could not find differences in genetic load among the deceptive orchid species *Himantoglossum robertianum* (Loisel) P.Delforge (syn. *Barlia robertiana*) and *A. morio* and the rewarding species *Anacamptis fragrans* (Pollini) R.M.Bateman.

Because pollinator behaviour is directly related to nectar availability it can be expected that both fitness and genetic consequences of a decreased population size are mediated by

the production of nectar reward. Deceptive orchids may be less prone to the loss of genetic variation from small populations because lack of floral reward may maintain outcrossing. Nectar production, on the other hand, may exacerbate the detrimental genetic consequences of small population size, as it reduces pollen movement among and within populations. So far, a systematic review of the relation between nectar production and susceptibility to the consequences of decreased population size is lacking. Here we reviewed all published studies that reported the relationship between orchid population size and population genetic diversity or fruit set. We used meta-analytical techniques to answer the following questions: (i) are fruit set and population genetic diversity in orchid species positively correlated with population size?; (ii) is this correlation stronger for rewarding orchid species than for deceptive species?; and (iii) do populations of deceptive orchid species exhibit lower among population genetic differentiation than populations of rewarding species?

MATERIALS AND METHODS

Data collection

In March 2013, we conducted a search in the Thomson Reuters Web of Science (ISI) database. We used a combination of the following keywords: *orchid*AND genetic* OR fruit set**. We supplemented the search with relevant studies cited in the first papers. We only included papers that reported either population genetic diversity or measures of fruit set together with population sizes and the sample size per population. Fruit set was recorded as the percentage of flowers that set fruit under natural conditions. The following measures of population genetic diversity were retrieved from each study when available: number of alleles per locus (A), the percentage of polymorphic loci (P), expected heterozygosity (H_e) and the inbreeding coefficient (F_{IS}). In some cases, F_{IS} was calculated as one minus the ratio between observed and expected heterozygosity. It is common practice to include the percentage of polymorphic loci in meta-analyses. However, the screening and selecting for polymorphic loci can differ between studies, and hence some reservation is necessary when interpreting the results. We also retrieved the genetic variation among populations (F_{ST}). When fruit set or genetic diversity values were not reported, we extracted raw data from graphs and tabulated it using GetData Graph Digitizer 2.24 (Fedorov 2008). The presence of nectar reward for each species was either mentioned in the published papers or retrieved from monographs (e.g. Claessens & Kleyen 2011). To exclude the possibility that results were dependent on the genetic markers applied, we also retrieved the type of marker used in each study. All studies used neutral genetic markers to investigate population genetic variation. Since these are likely not loci under selection, loss of neutral genetic variation does not always result in decreased fitness. Previous meta-analyses, however, showed that, in general, neutral genetic variation does correlate with population fitness (Reed & Frankham 2003, Leimu et al. 2006).

Data analysis

Effect sizes and their 95% bias corrected confidence intervals were calculated as the Spearman rank correlation coefficient (r_s) between population size and the different measures of population genetic diversity (A , P , H_e , F_{IS}) or fruit set (electronic appendix 1). We did not perform a Fisher z transformation on the individual correlation coefficients because this leads to more biased effect sizes (Field 2001). A positive effect size implies that population genetic diversity or fruit set declines with decreasing population size, except for F_{IS} where a positive effect size indicates lower homozygosity in small populations. Every study reported a single F_{ST} value as measure of genetic variation among studied populations; a high F_{ST} value implies more genetic drift and low gene flow. We treated the F_{ST} value of each study as an effect size in the analyses. Each study was weighted according to $[(A - 2)N]^{1/2}$, where A is the number of populations studied, and N is the mean number of individuals sampled across all populations (Reed & Frankham 2003).

When manuscripts with small treatment effects or non-significant results do not get published, the literature can become biased (Thornton & Lee 2000). This implies that there is a correlation between effect size and study weight as only large effects are reported by small sample size studies. To explore the possibility of publication bias, we constructed funnel plots by plotting effect sizes against study weights, and we tested the significance of this relationship using a Spearman rank correlation (Palmer 1999).

The shared phylogenetic history of taxonomic data can create biased results, as more related species may have similar traits and similar responses to environmental changes compared to distantly related species (Gitzendanner & Soltis 2000). Therefore, we tested for phylogenetic independence with phyloMeta version 1.3 (Lajeunesse 2009). We constructed a phylogenetic tree of the studied species in Mesquite 2.72 (Madison & Madison 2009) using phylogenetic data from Cameron et al. (1999) and Bateman et al. (2003) (electronic appendix 2). Because estimates of divergence times were unavailable, we applied four arbitrary branch-length assumptions: (1) all branch lengths equal 1; (2) branch length equals the number of species in a clade minus one with all the species constrained to be contemporaneous (Grafen 1989); (3) species are contemporaneous, but all internode branch lengths are equal to one (Pagel 1992); and (4) branch length from the tip to a current node equals the logarithm (base 10) of the number of tip species descending from that node (Nee's method) (Purvis 1995). To allow comparisons among the different phylogenetic and phylogenetically independent, traditional models, we calculated log-likelihood values and Akaike's information criterion (AIC) for each phylogenetic model. The model with the best fit was selected based on the Akaike weight (Burnham & Anderson 2001), which is the likelihood of the model divided by the sum of the likelihoods of all models.

We used MetaWin 2.1 software (Rosenberg 2000) for the meta-analysis and performed random-effects models. In contrast with fixed models, random models take into account that the dataset contains different species and different study areas (Borenstein et al. 2010). The heterogeneity

among effect sizes was assessed using the Q statistic (Hedges & Olkin 1985). When the Q statistic was significant, we explored its cause, using nectar reward (Yes or No) (Lipsey & Wilson 2001). In addition to Q statistics, we calculated 95% bias-corrected bootstrap confidence intervals (9999 iterations) for the overall effect size (Adams et al. 1997).

To exclude potential biases we performed additional weighted linear mixed models. We related the weighted effect size of the different measures to the fixed categorical predictor: nectar reward (Yes or No). The variables: smallest and largest population size, genetic marker (RAPD, AFLP, SSR, ISSR, isozyme or allozyme) and study were included as a random-variables in a set of different models. The models provided similar results and did not provide additional information (electronic appendix 3), therefore only the results of the classical meta-analysis are reported.

RESULTS

Generalities of the meta-analysis

We retrieved 28 studies on 26 different orchid species that reported one or more genetic diversity measures in relationship to population size, yielding a total of 32 records (see electronic appendix 1). Eleven species were rewarding, fourteen deceptive, and 1 had an unknown pollination system. We also retrieved sixteen studies reporting the relationship between population size and fruit set of seven rewarding and eleven deceptive orchid species, resulting in a total of 22 records (electronic appendix 1). Eighty-eight percent of the species analysed were temperate and terrestrial, but tropical or epiphytic orchids were also included. All studied species were entomophilous and all species were self-compatible, except one (*Anacamptis palustris* (Jacq.) R.M.Bateman, Pridgeon & M.W.Chase), which we could not find information on the mating system. This implies that mating system (self-incompatible vs. self-compatible) could not confound our results. The number of populations per study are reported in electronic appendix 1. There was no evidence of a publication bias as all funnel plots were funnel shaped and symmetrical, and none of the corresponding Spearman rank correlations were significant ($r_A = 0.29$, $r_P = -0.13$, $r_{H_e} = -0.08$, $r_{FIS} = -0.31$, $r_{\text{fruit set}} = -0.15$; $P > 0.15$ in all cases) (see electronic appendix 4). For all effect sizes, the best model fit was obtained when excluding phylogenetic relationship (see electronic appendix 5). Therefore, a traditional standard random-effects model was used in all subsequent analyses (Rosenberg 2000).

Consequences of small population size

Most spearman rank correlation effect sizes were all positive, indicating that orchid genetic diversity and fruit set decreased with decreasing population size ($P < 0.05$; fig. 1). F_{IS} however, did not significantly change with population size. Orchid pollination system (rewarding vs. deceptive) significantly affected the relationship between population size and fruit set ($Q_{\text{between}} = 5.36$, $P < 0.05$; electronic appendix 3, fig. 2). Fruit set of deceptive orchids was unaffected by population size whereas fruit set of rewarding orchids decreased with decreasing population size. Effect sizes calcu-

lated for the different measures of genetic diversity did not significantly differ between rewarding and deceptive orchids (electronic appendix 3, fig. 2). F_{ST} values of rewarding orchids were, however, significantly higher than F_{ST} values of deceptive orchid species (fig. 3; mean weighted $F_{ST} = 0.24$ and 0.10 for rewarding and deceptive orchids, respectively; $Q_{between} = 2.05$, $P < 0.05$). This suggests higher gene flow among populations of deceptive orchids, compared to rewarding species.

DISCUSSION

Our results show that fruit set significantly diminished with smaller population size. However, the effect of population size on fruit set was largely dependent on pollination system, with rewarding orchids showing a strongly positive correlation between fruit set and population size, whereas no such relation was found in deceptive species. Previous studies (Neiland & Wilcock 1998, Tremblay et al. 2005) showed that fruit set is higher in rewarding orchids compared to deceptive orchids, and a simple t-test on the original data of this study confirmed these findings ($P < 0.05$). For rewarding orchids, small population size thus appears to result in pollen limitation, through a reduction of available mating partners or pollinators, or both (Steffan-Dewenter & Tschamtkke 1999), or through changed plant-pollinator interactions (Kolb 2008, Pellissier et al. 2012). Alternatively, deceptive orchids may always be pollen limited due to pollinator learning, independent of population size (Neiland

& Wilcock 1998, Tremblay et al. 2005). Additionally, pollen limitation in small populations of deceptive orchids can be partly countered by rare-morph advantages (Smithson & Macnair 1997, Eckhart et al. 2006), leaving deceptive orchids fruit set unaffected by population size.

We were unable to differentiate between the effects of food and sexual deception as only three sexual deceptive orchids (three *Ophrys* species) were included in the dataset. It is hypothesized that the reproductive success of sexual deceptive orchids may be higher at small population sizes as they have a higher chance to be pollinated by the limited pool of misguided pollinators actively seeking out these flowers (Peakall & Beattie 1996). Our data confirms this hypothesis as these three studies show a strong negative effect size regarding the relation between population size and fruit set (see electronic appendix 1). Furthermore, by removing the sexually deceptive orchids from the analyses, the difference between nectar producing and food deceptive orchids lost significance, even though the general trend remains strong, and fruit set of deceptive orchids is unaffected by population size. ($Q_{between} = 2.7$, $P = 0.12$; $r_{fruitset} = 0,18$ and $0,47$ for food deceptive and reward pollination, respectively).

In both deceptive and rewarding species, population genetic variation significantly decreased with decreasing population size. The effect of population size was strongest for

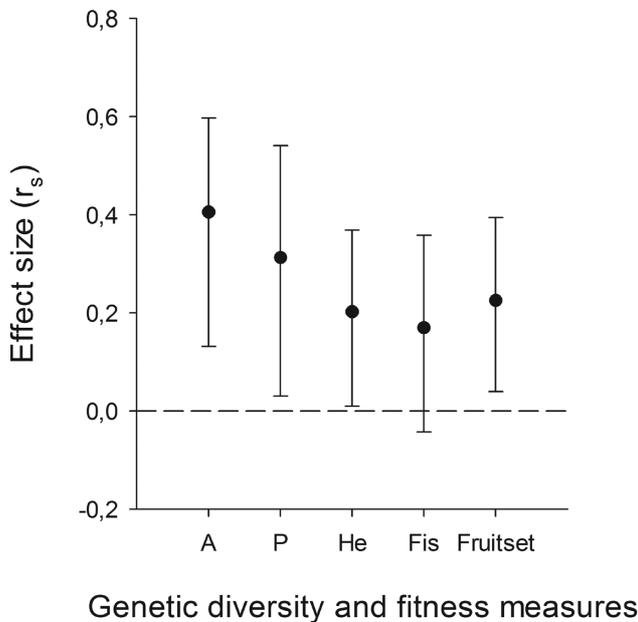


Figure 1 – Overall mean weighted effect sizes (Spearman rank correlations between genetic diversity and population size) and 95% bias-corrected bootstrap confidence intervals for four measures of genetic diversity in orchids (*A*, number of alleles per locus; *P*, percentage of polymorphic loci; *H_e*, expected heterozygosity; *F_{IS}*, inbreeding coefficient) and one fitness measure (fruit set).

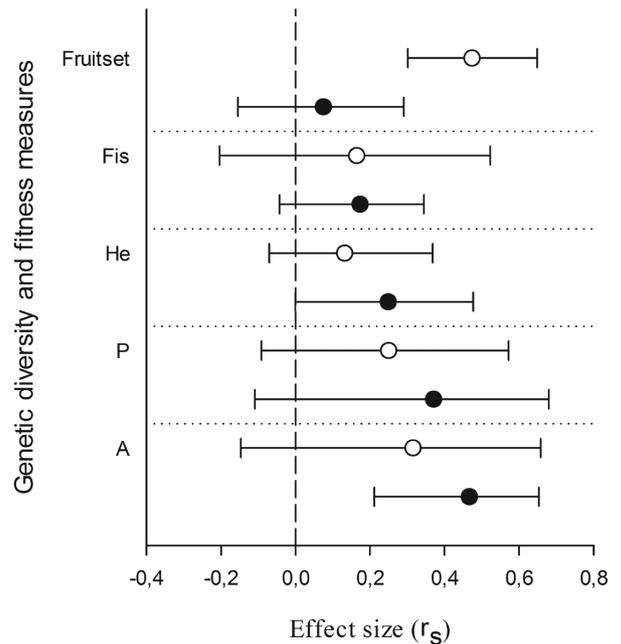


Figure 2 – Mean weighted effect sizes (Spearman rank correlations between population size and genetic diversity or fruit set) and 95% bias-corrected bootstrap confidence intervals calculated for rewarding (white squares) and deceptive (black squares) pollination in orchids. $Q_{between}$ statistics were significant for the fitness measure fruit set ($p < 0.05$), but not for the genetic diversity measures (F_{IS} , inbreeding coefficient; H_e , expected heterozygosity; P , percentage of polymorphic loci; A , number of alleles per locus; $p > 0.05$ in all cases).

the number of alleles per locus, suggesting that a reduction in population size will directly cause a reduced pool of alleles probably by eliminating those alleles that are present at low frequencies (mainly reflected by A) (Lowe et al. 2005). Subsequent generations will experience further reductions in genetic diversity (A , P and He) by the elimination of both rare and more common alleles through random genetic drift (Young et al. 1996). Compared to other plant species, orchids appear to be highly vulnerable to the loss of alleles. For example, the correlation effect size for A ($r_s = 0.41$) was stronger than that reported by Honnay & Jacquemyn (2007) ($r_s = 0.36$) who investigated habitat fragmentation effects in a much broader range of plant species. The effect size for P and He (0.31 and 0.20, respectively), on the other hand, were slightly lower than the values found by Honnay & Jacquemyn (2007) (0.35 and 0.23, respectively).

Similar to previous meta-analyses (Leimu et al. 2006, Honnay & Jacquemyn 2007, Aguilar et al. 2008) we found no relation relating F_{IS} to population size. A possible reason for a lack of a relationship between F_{IS} and population size is a selection process against less fit homozygotes in small orchid populations. Because environmental conditions in small habitats are often less suitable (e.g. through shrub encroachment in the case of grassland species or canopy closure in case of forest floor species), seedling recruitment is limited or non-existing, while only the most fit, least inbred individuals survive over time. Hence small populations only contain fit remnant individuals of large populations, while large populations also contain inbred seedlings. Additionally, due to loss of genetic diversity in small populations, F_{IS} can decrease because homozygotes of rare alleles are missing (Young et al. 1996). Interestingly, Angeloni et al. (2011), who directly measured inbreeding depression, found a positive correlation between the degree of inbreeding depression and population size. They suggested that it is possible that genetic load is purged in small populations, although Byers & Waller (1999) generally found only weak evidence for purging in plant populations.

We expected that deceptive orchids were better protected against the loss of genetic variation in small populations than rewarding species, for which nectar production might exacerbate the detrimental effects. Our results did not meet this expectation as the effect of decreasing population size on genetic diversity was not significantly different between deceptive and rewarding orchid species. This result is somewhat surprising, especially since the F_{ST} values suggested significantly higher gene flow among populations of deceptive orchids, compared to rewarding orchids, confirming earlier results of Cozzolino & Widmer (2005). Gene exchange among populations results in the supplementation of alleles lost in a particular population. However, when recent habitat fragmentation and degradation decrease the size of orchid populations, their isolation increases simultaneously (Andrén 1994). This likely reduces gene flow between populations and restricts the replenishment of lost alleles. Gene flow among populations of rewarding orchids is in general lower than in deceptive orchids as is shown by their relatively higher F_{ST} . Following increasing isolation, the population genetic diversity of rewarding orchids may therefore be less affected by reduced gene flow, as compared to deceptive spe-

cies that rely on a frequent replenishment of alleles to maintain their population genetic variation. This is similar to the way that the population genetic diversity of selfing species is less affected by habitat fragmentation compared to outcrossing species (Leimu et al. 2006, Honnay & Jacquemyn 2007). Thus, contrary to our expectations, we found that deceptive orchids were as susceptible to loss of genetic variation in small populations as rewarding orchids, potentially due to the simultaneous occurrence of increased population isolation.

To conclude, we found that both fruit set and genetic variation of orchids significantly declined in small populations. However, fruit set was, in general, not related to population size in deceptive orchids, whereas fruit set was significantly lower in smaller populations of rewarding species. Deceptive orchids, however, lost genetic variation from small populations to the same extent as rewarding species. Lower F_{ST} values in deceptive orchids further indicated that gene flow among populations is higher in deceptive than in rewarding species. Therefore, decreasing population sizes may have a stronger effect on population genetic diversity of deceptive orchids if gene flow is reduced. Furthermore, F_{IS} showed no relation with population size, potentially due to a selection process against less fit homozygotes in small orchid populations. Thus, our results indicate that small population sizes strongly diminish the population viability of both rewarding and non-rewarding orchids. Fitness of rewarding orchids mainly declines through ecological mechanisms, whereas deceptive orchids seem to be more susceptible to loss of genetic diversity when gene flow becomes reduced.

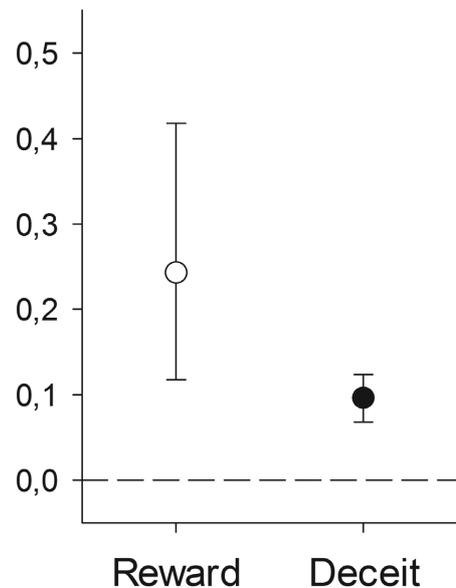


Figure 3 – Comparison of weighted mean genetic differentiation (F_{ST}) among deceptive and rewarding orchid populations. Rewarding orchid populations have significantly higher F_{ST} than deceptive orchid populations ($Q_{\text{between}} = 2.05$, $p < 0.05$, bars represent 95% bias-corrected bootstrap confidence intervals).

SUPPLEMENTARY DATA

Supplementary data are available in pdf at *Plant Ecology and Evolution*, Supplementary Data Site (<http://www.ingentaconnect.com/content/botbel/plecevo/supp-data>) and consist of: (1) orchid species and studies used for the meta-analysis; (2) phylogenetic tree of all the orchid species used in the meta-analyses; (3) results of traditional meta-analysis and additional weighted mixed-model analysis; (4) funnel plots of effect size using the Spearman rank correlation coefficient (r) versus study weight for four genetic diversity measures and fruitset; and (5) results of Akaike information criterion (AIC) analyses for five evolutionary random-effects models of plant phylogeny.

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REFERENCES

- Adams D.C., Gurevitch J., Rosenberg M.S. (1997) Resampling tests for meta-analysis of ecological data. *Ecology* 78: 1277–1283. [http://dx.doi.org/10.1890/0012-9658\(1997\)078\[1277:RT-FMAO\]2.0.CO;2](http://dx.doi.org/10.1890/0012-9658(1997)078[1277:RT-FMAO]2.0.CO;2)
- Ågren J. (1996) Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria*. *Ecology* 77: 1779–1790. <http://dx.doi.org/10.2307/2265783>
- Aguilar R., Quesada M., Ashworth L., Herrerias-Diego Y., Lobol J. (2008) Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. *Molecular Ecology* 17: 5177–5188. <http://dx.doi.org/10.1111/j.1365-294X.2008.03971.x>
- Andrén H. (1994) Effects of habitat fragmentation on birds and mammals in landscapes with different proportions of suitable habitat: a review. *Oikos* 71: 355–366. <http://dx.doi.org/10.2307/3545823>
- Angeloni F.N., Ouborg J., Leimu R. (2011) Meta-analysis on the association of population size and life history with inbreeding depression in plants. *Conservation Biology* 144: 35–43. <http://dx.doi.org/doi:10.1016/j.biocon.2010.08.016>
- Bateman R.M., Hollingsworth P.M., Preston J., Luo Y.B., Pridgeon A.M., Chase M.W. (2003) Molecular phylogenetics and evolution of Orchidinae and selected Habenariinae (Orchidaceae). *Botanical Journal of the Linnean Society* 142: 1–40. <http://dx.doi.org/10.1046/j.1095-8339.2003.00157.x>
- Booy G., Hendriks R.J.J., Smulders M.J.M., Van Groenendael J.M., Vosman B. (2000) Genetic diversity and the survival of populations. *Plant Biology* 2: 379–395. <http://dx.doi.org/10.1055/s-2000-5958>
- Borenstein M., Hedges L.V., Higgins J.P.T., Rothstein H.R. (2010) A basic introduction to fixed-effect and random-effects models for meta-analysis. *Research Synthesis Methods* 1: 97–111. <http://dx.doi.org/10.1002/jrsm.12>
- Burnham K.P., Anderson D.R. (2001) Kullback-Leibler information as a basis for strong inference in ecological studies. *Wildlife Research* 28: 111–119. <http://dx.doi.org/10.1071/WR99107>
- Byers D.L., Waller D.M. (1999) Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. *Annual Review Ecology and Systematics* 30: 479–513. <http://dx.doi.org/10.1146/annurev.ecolsys.30.1.479>
- Cameron K.M., Chase M.W., Whitten W.M., Kores P.J., Jarrell D.C., Albert V.A., Yukawa T., Harold G.H., Goldman D.H. (1999) A phylogenetic analysis of the Orchidaceae: evidence from rbcL nucleotide sequences. *American Journal of Botany* 86: 208–224. <http://dx.doi.org/10.2307/2656938>
- Chase M.W. (2005) Classification of Orchidaceae in the age of DNA data. *Curtis's Botanical Magazine* 22: 2–7. <http://dx.doi.org/10.1111/j.1355-4905.2005.00466.x>
- Charlesworth D., Willis J.H. (2009) The genetics of inbreeding depression. *Nature Reviews Genetics* 10: 783–796. <http://dx.doi.org/10.1038/nrg2664>
- Claessens J., Kleynen J. (2011) The flower of the European orchid. Form and function. Voerendaal/Stein, Schrijen-Lippertz.
- Cozzolino S., Widmer A. (2005) Orchid diversity: an evolutionary consequence of deception? *Trends in Ecology & Evolution* 20: 487–494. <http://dx.doi.org/10.1016/j.tree.2005.06.004>
- Dornier A., Cheptou P.O. (2012) Determinants of extinction in fragmented plant populations: *Crepis sancta* (asteraceae) in urban environments. *Oecologia* 169: 703–712. <http://dx.doi.org/10.1007/s00442-011-2229-0>
- Dressler R. (1981) *The Orchids - Natural History and Classification*. Harvard, Harvard University Press.
- Eckhart V.M., Rushing N.S., Hart G.M., Hansen J.D. (2006) Frequency-dependent pollinator foraging in polymorphic *Clarkia xantiana* ssp. *xantiana* populations: implications for flower colour evolution and pollinator interactions. *Oikos* 112: 412–421. <http://dx.doi.org/10.1111/j.0030-1299.2006.14289.x>
- Fedorov S. (2008) GetData graph digitizer. Version 2.24. Kogarah, Australia, GetData.
- Field A.P. (2001) Meta-analysis of correlation coefficients: a Monte Carlo comparison of fixed- and random-effects methods. *Psychological Methods* 6: 161–180. <http://dx.doi.org/10.1037/1082-989X.6.2.161>
- Gitzendanner M.A., Soltis P.S. (2000) Patterns of genetic variation in rare and widespread congeners. *American Journal of Botany* 87: 783–792. <http://dx.doi.org/10.2307/2656886>
- Grafen A. (1989) The phylogenetic regression. *Philosophical Transactions of the Royal Society B: Biological Sciences* 326: 119–157. <http://dx.doi.org/10.1098/rstb.1989.0106>
- Hedges L.V., Olkin I. (1985) *Statistical methods for meta-analysis*. California, Academic Press.
- Honnay O. (2013) Genetic Drift. In: Maloy S., Hughes K. (eds) *Brenner's Encyclopedia of Genetics*: 251–253. 2nd Ed. Oxford, Elsevier. <http://dx.doi.org/10.1016/B978-0-12-374984-0.00616-1>
- Honnay O., Jacquemyn H. (2007) Susceptibility of common and rare plant species to the genetic consequences of habitat fragmentation. *Conservation Biology* 21: 823–831. <http://dx.doi.org/10.1111/j.1523-1739.2006.00646.x>
- Jersáková J., Johnson S.D., Kindlmann P. (2006) Mechanisms and evolution of deceptive pollination in orchids. *Biological Reviews* 81: 219–235. <http://dx.doi.org/10.1017/S1464793105006986>
- Jersáková J., Johnson S.D., Kindlmann P., Pupin A.C. (2008) Effect of nectar supplementation on male and female components of pollination success in the deceptive orchid *Dactylorhiza sambucina*. *Acta Oecologica* 33: 300–306. <http://dx.doi.org/10.1016/j.actao.2008.01.001>

- Johnson S.D. (2000) Batesian mimicry in the non-rewarding orchid *Disa pulchra*, and its consequences for pollinator behaviour. *Biological Journal of the Linnean Society* 71: 119–132. <http://dx.doi.org/10.1111/j.1095-8312.2000.tb01246.x>
- Johnson S.D., Craig I.P., Ågren J. (2004) The effects of nectar addition on pollen removal and geitonogamy in the non-rewarding orchid *Anacamptis morio*. *Proceedings of the Royal Society: B Biological Sciences* 271: 803–809. <http://dx.doi.org/10.1098/rspb.2003.2659>
- Keller L.F., Waller D.M. (2002) Inbreeding effects in wild populations. *Trends in Ecology and Evolution* 17: 230–241. [http://dx.doi.org/10.1016/S0169-5347\(02\)02489-8](http://dx.doi.org/10.1016/S0169-5347(02)02489-8)
- Kolb A. (2008) Habitat fragmentation reduces plant fitness by disturbing pollination and modifying response to herbivory. *Biological conservation* 141: 2540–2549. <http://dx.doi.org/10.1016/j.biocon.2008.07.015>
- Lajeunesse M.J. (2009) Meta-analysis and the comparative phylogenetic method. *The American Naturalist* 174: 369–381. <http://dx.doi.org/10.1086/603628>
- Leimu R., Mutikainen P., Koricheva J., Fischer M. (2006) How general are positive associations between population size, fitness, and genetic variation? *Journal of Ecology* 94: 942–952. <http://dx.doi.org/10.1111/j.1365-2745.2006.01150.x>
- Lipsey M.W., Wilson D.B. (2001) *Practical Meta-Analysis*. Thousand Oaks, Sage Publications.
- Lowe A.J., Boshier D., Ward M., Bacles C.F.E., Navarro C. (2005) Genetic resource impacts of habitat loss and degradation; reconciling empirical evidence and predicted theory for neotropical trees. *Heredity* 95: 255–273. <http://dx.doi.org/10.1038/sj.hdy.6800725>
- Maddison W.P., Maddison D.R. (2009) *Mesquite: a modular system for evolutionary analysis*. Version 2.72. Available from <http://mesquiteproject.org/> [accessed 3 Dec. 2014].
- Neiland M.R.M., Wilcock C.C. (1998) Fruit set, nectar reward, and rarity in the Orchidaceae. *American Journal of Botany* 85: 1657–1671. <http://dx.doi.org/10.2307/2446499>
- Nilsson L.A. (1992) Orchid pollination biology. *Trends in Ecology & Evolution* 7: 255–259. [http://dx.doi.org/10.1016/0169-5347\(92\)90170-G](http://dx.doi.org/10.1016/0169-5347(92)90170-G)
- Pagel M.D. (1992) A method for the analysis of comparative data. *Journal of Theoretical Biology* 156: 431–442. [http://dx.doi.org/10.1016/S0022-5193\(05\)80637-X](http://dx.doi.org/10.1016/S0022-5193(05)80637-X)
- Palmer A.R. (1999) Detecting publication bias in meta-analysis: a case study of fluctuating asymmetry and sexual selection. *The American Naturalist* 154: 220–233. <http://dx.doi.org/10.1086/303223>
- Peakall R., Beattie A.J. (1996) Ecological and genetic consequences of pollination by sexual deception in the orchid *Caladenia tentaculata*. *Evolution* 50: 2207–2220. <http://dx.doi.org/10.2307/2410692>
- Pellissier V, Muratet A, Verfaillie F, Machon N. (2012) Pollination success of *Lotus corniculatus* (L.) in an urban context. *Acta Oecologica* 39: 94–100. <http://dx.doi.org/10.1016/j.actao.2012.01.008>
- Purvis A. (1995) A composite estimate of primate phylogeny. *Philosophical Transactions of the Royal Society: B Biological Sciences* 348: 405–421. <http://dx.doi.org/10.1098/rstb.1995.0078>
- Rasmussen H.N., Rasmussen F.N. (2009) Orchid mycorrhiza: implications of a mycophagous life style. *Oikos* 118: 334–345. <http://dx.doi.org/10.1111/j.1600-0706.2008.17116.x>
- Reed D.H., Frankham R. (2003) Correlation between fitness and genetic diversity. *Conservation Biology* 17: 230–237. <http://dx.doi.org/10.1046/j.1523-1739.2003.01236.x>
- Rosenberg M.S. (2000) *MetaWin: statistical software for meta-analysis*. Version 2.1. Sunderland, Massachusetts, Sinauer Associates.
- Schleuning M., Niggemann M., Becker U., Matthies D. (2009) Negative effects of habitat degradation and fragmentation on the declining grassland plant *Trifolium montanum*. *Basic and Applied Ecology* 10: 61–69. <http://dx.doi.org/10.1016/j.baae.2007.12.002>
- Smith S.E., Read D.J. (2008) *Mycorrhizal Symbioses*. London, Academic Press.
- Smithson A., Macnair M.R. (1997) Negative frequency dependent selection by pollinators on artificial flowers without reward. *Evolution* 51: 715–723. <http://dx.doi.org/doi:10.2307/2411148>
- Smithson A. (2006) Pollinator limitation and inbreeding depression in orchid species with and without nectar rewards. *New Phytologist* 169: 419–430. <http://dx.doi.org/10.1111/j.1469-8137.2005.01592.x>
- Steffan-Dewenter I., Tschardt T. (1999) Effects of habitat isolation on pollinator communities and seed set. *Oecologia* 121: 432–440. <http://dx.doi.org/10.1007/s004420050949>
- Swarts N.D., Dixon K.W. (2009) Terrestrial orchid conservation in the age of extinction. *Annals of Botany* 104: 543–556. <http://dx.doi.org/10.1093/aob/mcp025>
- Thornton A., Lee P. (2000) Publication bias in meta-analysis: its causes and consequences. *Journal of Clinical Epidemiology* 53: 207–216. [http://dx.doi.org/10.1016/S0895-4356\(99\)00161-4](http://dx.doi.org/10.1016/S0895-4356(99)00161-4)
- Tremblay R.L., Ackerman J.D., Zimmerman J.K., Calvo R.N. (2005) Variation in sexual reproduction in orchids and its evolutionary consequences: a spasmodic journey to diversification. *Biological Journal of the Linnean Society* 84: 1–54. <http://dx.doi.org/10.1111/j.1095-8312.2004.00400.x>
- van der Cingel N.A. (1995) *An Atlas of Orchid Pollination: European Orchids*. Rotterdam, A.A. Balkema.
- van der Pijl L., Dodson C.H. (1966) *Orchid Flowers: their pollination and evolution*. Miami, University of Miami Press.
- Young A., Boyle T., Brown T. (1996) The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology & Evolution* 11: 413–418. [http://dx.doi.org/10.1016/0169-5347\(96\)10045-8](http://dx.doi.org/10.1016/0169-5347(96)10045-8)
- Young A.G., Clarke G.M. (2000) *Genetics, demography and viability of fragmented populations*. Cambridge, Cambridge University Press.

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