Fluctuating selection across years and phenotypic polymorphism in food-deceptive orchids (#17959)

First submission

Please read the **Important notes** below, the **Review guidance** on page 2 and our **Standout reviewing tips** on page 3. When ready **submit online**. The manuscript starts on page 4.

Important notes

Editor and deadline

David Roberts / 9 Jun 2017

Files	2 Figure file(s) 2 Table file(s) 1 Raw data file(s) 1 Other file(s) Please visit the overview page to **download and review** the files not included in this review PDF.
Declarations	No notable declarations are present

Summary of Comments on peerj-reviewing-17959-v0 JDArev.pdf

This page contains no comments

Review guidelines

Please read in full before you begin

How to review

When ready **submit your review online**. The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

You can also annotate this PDF and upload it as part of your review

To finish, enter your editorial recommendation (accept, revise or reject) and submit.

BASIC REPORTING

- ✓ Clear, unambiguous, professional English language used throughout.
- ✓ Intro & background to show context. Literature well referenced & relevant.
- ✓ Structure conforms to **PeerJ standards**, discipline norm, or improved for clarity.
- ✓ Figures are relevant, high quality, well labelled & described.
- ✓ Raw data supplied (see **PeerJ policy**).

EXPERIMENTAL DESIGN

- ✓ Original primary research within **Scope of the journal**.
- ✓ Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
- ✓ Rigorous investigation performed to a high technical & ethical standard.
- ✓ Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

- ℹ Impact and novelty not assessed. Negative/inconclusive results accepted. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
- ✓ Data is robust, statistically sound, & controlled.
- ✓ Conclusions are well stated, linked to original research question & limited to supporting results.
- ✓ Speculation is welcome, but should be identified as such.

The above is the editorial criteria summary. To view in full visit **https://peerj.com/about/editorial-criteria/**

This page contains no comments

7 Standout reviewing tips

The best reviewers use these techniques

Tip	*Example*
Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
Give specific suggestions on how to improve the manuscript	*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*
Comment on language and grammar issues	*The English language should be improved to ensure that your international audience can clearly understand your text. I suggest that you have a native English speaking colleague review your manuscript. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult.*
Organize by importance of the issues, and number your points	*1. Your most important issue* *2. The next most important item* *3. …* *4. The least important points*
Give specific suggestions on how to improve the manuscript	*Line 56: Note that experimental data on sprawling animals needs to be updated. Line 66: Please consider exchanging "modern" with "cursorial".*
Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

This page contains no comments

Fluctuating selection across years and phenotypic polymorphism in food-deceptive orchids

Giovanni Scopece Corresp., [1] , **Nicolas Juillet** [2] , **Christian Lexer** [3] , **Salvatore Cozzolino** [1]

[1] Department of Biology, University of Naples Federico II, Naples, Italy
[2] UMR Peuplements Végétaux et Bioagresseurs en Milieu Tropical, Université de la Réunion, Pôle de Protection des Plantes, Saint Pierre, La Réunion, France
[3] Department of Botany and Biodiversity Research, University of Vienna, Vienna, Austria

Corresponding Author: Giovanni Scopece
Email address: giovanni.scopece@unina.it

Pollinator-mediated selection is predicted to be among the most important mechanisms determining floral trait evolution in entomophilous species. Orchids are widely used as models to study asymmetric plant-insect interactions because they encompass several thousand species with nectarless flowers that deceive nectar-searching pollinators. High levels of intra-specific phenotypic polymorphism have been reported in deceptive orchids, suggesting a reduced action of pollinator-mediated selection on their floral traits. Nevertheless, several studies report on widespread directional selection mediated by pollinators even in these deceptive orchids. In this study we test the hypothesis that the observed selection can fluctuate across years in strength and direction thus explaining the phenotypic variability of this orchid group. We performed a three-year study estimating selection differentials and selection gradients for nine phenotypic traits involved in insect attraction in two Mediterranean orchid species, namely *Orchis mascula* and *O. pauciflora*, both relying on a well-described food-deceptive pollination strategy. We found overall weak directional selection and marginally significant selection gradients in the two investigated species with significant intra-specific differences in selection differentials across years. Although our data do not link this variation with a specific environmental cause, our results suggest that pollinator-mediated selection in food-deceptive orchids can change in strength and in direction over time. In perennial plants, such as orchids, different selection differentials in the same populations in different flowering seasons can contribute to the maintenance of phenotypic polymorphism often reported in deceptive orchids.

This page contains no comments

Fluctuating selection across years and phenotypic polymorphism in food-deceptive orchids

Short title: Fluctuating selection in orchids

Giovanni Scopece [1], Nicolas Juillet [1, 2], Christian Lexer [3], Salvatore Cozzolino [1]

[1] Department of Biology, University Federico II, via Cinthia, I-80126, Naples, Italy

[2] UMR Peuplements Végétaux et Bioagresseurs en Milieu Tropical, Université de la Réunion, Pôle de Protection des Plantes, 97410 Saint Pierre, La Réunion

[3] University of Vienna, Department of Botany and Biodiversity Research, Rennweg 14, A-1030 Vienna, Austria

Correspondence
G. Scopece, Department of Biology, University of Naples Federico II, via Cinthia, I-80126, Naples, Italy. Tel.: +39081679186; E-mail: giovanni.scopece@unina.it

This page contains no comments

ABSTRACT

Pollinator-mediated selection is predicted to be among the most important mechanisms determining floral trait evolution in entomophilous species. Orchids are widely used as models to study asymmetric plant-insect interactions because they encompass several thousand species with nectarless flowers that deceive nectar-searching pollinators. High levels of intra-specific phenotypic polymorphism have been reported in deceptive orchids, suggesting a reduced action of pollinator-mediated selection on their floral traits. Nevertheless, several studies report on widespread directional selection mediated by pollinators even in these deceptive orchids. In this study we test the hypothesis that the observed selection can fluctuate across years in strength and direction thus explaining the phenotypic variability of this orchid group.

We performed a three-year study estimating selection differentials and selection gradients for nine phenotypic traits involved in insect attraction in two Mediterranean orchid species, namely *Orchis mascula* and *O. pauciflora*, both relying on a well-described food-deceptive pollination strategy.

We found overall weak directional selection and marginally significant selection gradients in the two investigated species with significant intra-specific differences in selection differentials across years.

Although our data do not link this variation with a specific environmental cause, our results suggest that pollinator-mediated selection in food-deceptive orchids can change in strength and in direction over time. In perennial plants, such as orchids, different selection differentials in the same populations in different flowering seasons can

This page contains no comments

contribute to the maintenance of phenotypic polymorphism often reported in deceptive orchids.

Keywords: food-deceptive orchids; floral traits; fluctuating selection; *Orchis mascula*; *Orchis pauciflora*; phenotypic polymorphism; selection differentials.

This page contains no comments

INTRODUCTION

Pollinator-mediated selection is one of the main forces driving the evolution of floral traits in entomophilous pollen-limited plant species (Fenster et al., 2004). The typical form of entomophilous pollination is based on a reciprocal advantage: the flower offers a reward (typically pollen or nectar) to the pollinator, which transports the pollen to conspecific individuals ensuring reproduction. However, pollinators are unable to directly assess the amount of nectar reward contained in the flowers and thus depend on the information provided by advertising floral traits (Benitez-Vieyra et al., 2010). Therefore, nectar-producing plants have an advantage in being easily recognisable by pollinators such that once a rewarding flower type is discovered, the pollinators usually concentrate on a single species (flower constancy; Waser, 1986). In these plant-pollinator relationships, plants are continuously exposed to the choice of pollinators, which imposes a selection on flower traits (Schiestl & Johnson, 2013). On the basis of this evidence, strong emphasis has traditionally been given to pollinator-mediated selection in shaping changes in phenotypic floral trait distributions (Fenster et al., 2004) and the development of easily applicable methods to estimate selection differentials in natural populations (Lande & Arnold, 1983), resulting in the common discovery of directional selection on floral traits in many plant systems (Kingsolver et al., 2001; Hereford et al., 2004).

Flower constancy is an important prerequisite for the establishment of strong selection (stabilising or directional); when insects concentrate on phenotypes that they can associate with a reward, they favour individuals that are more recognisable and potentially shape the displacement of the phenotypic distribution of floral traits via the

This page contains no comments

elimination of abnormal phenotypes (Waser, 1986). However, plant-pollinator interactions can be more intricate, and patterns of pollinator-mediated selection may differ in the case of asymmetric and non-constant relationships.

Orchids are widely used as a model to study asymmetric plant-insect interactions because they include many species with nectarless flowers that deceive their pollinators (Schiestl, 2005; Jersàkovà et al., 2006). Among orchid deceptive pollination strategies, the most common is based on a generalised mimicry of floral traits that pollinators associate with the presence of nectar (generalised food-deception, Dafni, 1984). In these deceptively pollinated species, plant-pollinator interactions do not follow the flower constancy behaviour because insects learn to avoid deceptive flowers after a few rewardless visits (Gumbert, 2000; Smithson & Gigord, 2003). This pollinator behaviour can result in a strong selection for high phenotypic variability or in a relaxed selection on floral traits (Juillet & Scopece, 2010) of deceptive orchids, which is also suggested by the high intra-specific phenotypic variability in flower traits that has been observed in this plant group, including colour (Nilsson 1980), shape and size (Ackerman & Galarza-Pérez, 1991), and fragrance (Moya & Ackerman, 1993; Salzmann et al., 2007). In a survey, Ackerman et al. (2011) confirmed that such polymorphism is more common in deceptive compared to nectar-rewarding species, thus suggesting that the maintenance of a high phenotypic variability may be linked with exploitation in deceptive pollination systems (see also Salzmann et al., 2007).

The reasons that underlie the maintenance of high levels of phenotypic polymorphism in deceptive systems were investigated in several studies (see Juillet & Scopece, 2010 and references therein), particularly focussing on the idea, proposed by Heinrich (1975),

Page: 9

Number: 1 Author: Subject: Sticky Note Date: 5/31/17, 4:36:05 PM -04'00'

How can there be selection for variability since we are usually treating variation as a population attribute, yet fitness is associated with individuals. Of course, there may be selection for phenotypic plasticity, though this is often disputed. As you point out, variability can be the consequence of selection, e.g., there can be either negative frequency dependent selection, disruptive selection, both result in increased variation. Fluctuating directional selection over time that also results in variability that phenotypic variability decreases pollinator avoidance learning, thereby increasing orchid reproductive success. Such hypothesis has been recently suggested by Stejskal et al. (2015) for explaining the variation in labellum pattern of sexually-deceptive orchids. However, Juillet and Scopece (2010) showed that all attempts to identify a reproductive advantage linked with polymorphism in food-deceptive species were non-significant or indicated the opposite pattern thus underlining the need of more studies to support or discard this hypothesis. Other potential causes that could account for higher phenotypic variation in non-rewarding species are negative frequency dependent selection (e.g. Gigord et al., 2001) or, indirectly, differences in demographic history through processes as gene flow, genetic drift and founder events (Knapp & Rice, 1998; Holderegger et al., 2006; Lawton-Rauh, 2008). However, the first was contradicted by several studies that excluded a reproductive advantage of the rarer phenotype (Juillet & Scopece, 2010 and references therein) and the second appears unlikely because polymorphism is a common characteristic of deceptive orchid populations and it is thus difficult to link with historic processes.

Nevertheless, despite high levels of phenotypic polymorphism, there is increasing evidence for directional selection on floral traits in food-deceptive orchids. For example, pollination success was found to be correlated with plant height in *Cypripedium acaule* (O'Connell & Johnston, 1998), to the number of flowers in *Anacamptis morio* (Johnson & Nilsson, 1999), to spur length in the *Disa draconis* species complex (Johnson & Steiner, 1997) and in hybrid zones between *Anacamptis morio* and *A. longicornu* (Zitari et al., 2012), to flowering time in some deceptive orchids (O'Connell & Johnston, 1998; Sun et al., 2009; but see Sletvold *et al.* 2010), to plant height, flower number and spur

Page: 10

Number: 1 Author: Subject: Sticky Note Date: 5/29/17, 11:35:12 PM -04'00'

Polymorphisms are usually regarded descrete morphotypes (e.g., white vs pink flowers), whereas variability in most deceptive species involves continuous traits. I would say instead that high phenotypic variation is typical of deceptive orchids (sometimes it does involve descrete polymorphisms)

Number: 2 Author: Subject: Sticky Note Date: 5/28/17, 9:35:03 PM -04'00'

disruptive selection differential for flowering time was reported in Sabat & Ackerman 1996 Am J Bot length in *Dactylorhiza lapponica* (Sletvold et al., 2010) and to flower brightness and contrast in *Anacamptis morio* (Sletvold et al., 2016). This evidence is unexpected, considering the high levels of phenotypic polymorphism seen in deceptive orchids and suggests that, even in this plant group, directional selection mediated by pollinators may be widespread and strong. However, these studies were performed in single flowering seasons and disregarded the fact that across years natural selection can vary in both the strength and direction (Darwin, 1859; Grant & Grant, 1989; Benitez-Vieyra et al., 2012; Sletvold & Ågren, 2014). Temporal variation in patterns of natural selection has been often documented in plants (Harder & Johnson, 2009) and has been linked to variation in the pollinator community (Conner et al., 2003), to the presence of herbivores (Sandring et al., 2007), and to abiotic factors (Maad, 2000; Caruso et al., 2003; Maad & Alexandersson, 2004). Despite this evidence, however, the incidence of seasonal variation on selection patterns in deceptive species has been poorly investigated.

By estimating the covariance of pollination success with nine different phenotypic traits, we estimated selection differentials and gradients in two Mediterranean food-deceptive orchid species, *Orchis mascula* and *Orchis pauciflora*. In particular, we estimated the strength and direction of natural selection over three consecutive years in a sympatric population of these two species with the aim of specifically understanding whether, in the same populations, pollinator-mediated selection shows a concordant pattern over different years. We used two orchid species with similar flower morphology and a common set of pollinators (Van der Cingel, 1995; Cozzolino et al., 2006; Nilsson, 2008; Valterovà et al., 2007) as replicates to increase the power of our conclusions.

Number: 1 Author: Subject: Sticky Note Date: 5/29/17, 10:42:10 PM -04'00'

I would not say poorly investigated, but rather "not thoroughly investigated" or "rarely investigated". See Tremblay et al. 2010. Riding across the selection landscape: fitness consequences of annual variation in reproductive characteristics. Phil. Trans. R. Soc. B 365: 491-498.

Number: 2 Author: Subject: Sticky Note Date: 5/31/17, 4:37:52 PM -04'00'

True, if you are interested in the genus Orchis, but a broader taxonomic sampling would improve the argument for deception orchids family-wide. While the focus is on deceptive orchids and their high levels of variation in floral traits, annual variation in environmental conditions and biotic interactions can also influence the strength and direction of selection. Such dynamics occur in other orchids and flowering plants in general, regardless of the reward-deception dichotomy. The Tremblay et al. 2010 paper helps in this regard (deceptive Caladenia and Tolumnia were used as examples).

MATERIALS AND METHODS

Study System

Orchis mascula and *O. pauciflora* are closely related species in the orchid subtribe Orchidinae (Aceto et al., 1999). *O. mascula* is a widespread European species, ranging from Sweden to the northern borders of the Mediterranean basin (Sundermann, 1980). It is typically found in sunny meadows or calcareous grasslands up to 2400 m in altitude. *O. pauciflora* is generally found on poorer calcareous soils in the south-eastern and central part of the Mediterranean basin, up to 1500 m in altitude.

The two species are self compatible but non autogamous and rely on generalised food-deception for pollinator attraction (Van der Cingel, 1995). Hymenopterans are the most common pollinators of these two species (specifically *Bombus* sp., but also *Psithi*[illegible], *Eucera*, *Andrena*, *Osmia*, *Anthophora*), and reproductive success is severely pollen-limited (e.g., Cozzolino et al., 2006). Clonal propagation is extremely rare in both species.

Our study was performed during Spring 2002, 2003 and 2004 in sympatric natural populations of *O. mascula* and *O. pauciflora* located in the Cilento and Vallo di Diano National Park (Southern Italy). For each species, and in each year, plants were randomly selected in subgroups in an area of approximately 4 km^2 where distribution was nearly continuous. We measured a total of 1188 individuals (492 *O. mascula* and 696 *O. pauciflora*; for details see Raw data).

Page: 12

Number: 1 Author: Subject: Sticky Note Date: 5/28/17, 2:30:22 PM -04'00'

Psithyrus

Morphological measurement and data collection

To investigate pollinator-mediated selection, we measured nine morphological traits that are potentially important for pollinator visual attraction. Morphological trait measurements were obtained on the same day and at a time when all the examined plants were at peak flowering. As flower traits have been reported to be slightly variable within orchid inflorescences with stronger deviations occurring most frequently in the lowermost and uppermost flowers (Bateman & Rudall, 2006), flowers were sampled from the mid point of the inflorescence. For each individual that was sampled in this study, we recorded two "inflorescence size" variables: 1) flower number and 2) plant height (from ground to uppermost flower, to the nearest cm). From the same set of individuals, we measured three "flower size" variables: 3) labellum width (distance between the edges of the two lateral lobes, to the nearest 0.1 mm), 4) labellum length (distance between the labellum tip and spur mouth, to the nearest 0.1 mm) and 5) spur length (distance between the spur mouth and the spur tip, to the nearest 0.1 mm). To obtain these measurements, sampled flowers were dissected and floral elements were placed between two transparent plastic film sheets (supplementary figure 1). These sheets were subsequently scanned to obtain digital images in a 300 dpi TIFF format with a coordinate millimetre paper on the back for reference; measures of floral traits were obtained using ImageJ 1.33 software (Rasband, National Institutes of Health, USA). Finally, as previously described by Bradshaw et al. (1998), we also measured the pigment content in flower elements using a spectrophotometric method on the same individuals used for morphometric analyses. Anthocyanin concentration (purple pigment) was estimated from tepals or labellum, extracting the anthocyanins with 0.5-ml methanol/0.1% HCl, and determining the

Page: 13

Number: 1 Author: Subject: Sticky Note Date: 5/28/17, 7:16:22 PM -04'00'

how do you define the mid point? half way up the rachis? The median flower position? something else?

Number: 2 Author: Subject: Sticky Note Date: 5/28/17, 7:13:32 PM -04'00'

do all flowers on the inflorescence flower simultaneously or is there sequential development? If the latter is the case, then did you wait until the last bud opened before obtaining inflorescence height?

absorbance at 510 nm; carotenoid concentration (yellow pigment) was estimated similarly, using methylene chloride for pigment extraction and measuring absorbance at 450 nm. We thus estimated four “flower colour” variables: 6) carotenoid content in tepals, 7) carotenoid content in labellum, 8) anthocyanins content in tepals and 9) anthocyanins content in labellum.

We estimated pollen limitation (PL) as 1 - (mean female fitness of open-pollinated plants/mean female fitness of hand pollinated plants), ranging from 0 to 1. Female fitness of open pollinated plants was recorded on the same individuals used for traits measurements and was defined as the number of fruits produced by an individual relative to its number of flowers; female fitness of hand pollinated plants was calculated similarly based on literature data reporting results of crossing experiments conducted in the same populations (Scopece et al., 2007).

Data analysis

All analyses were performed independently for the two species and were conducted using R 2.1.1 software (R Development Core Team 2005). To explore the relationship among floral traits, we performed Spearman’s rank correlation. We examined significant differences in floral traits across years using Kruskal-Wallis tests of difference and Tukey HSD posthoc comparison tests. To explore the level of variation of floral traits, for both investigated species, we calculated a coefficient of variation (CV) as the ratio between Standard deviation and mean. CVs were calculated for each trait in the three

This page contains no comments

investigated years and then averaged to obtain a value for each of the two species.

We estimated selection differentials (Lande & Arnold, 1983; Brodie et al., 1995) as the covariance between relative reproductive success and each morphological trait. Plant reproductive success was measured at the end of the flowering period as fruit set, i.e., number of fruit / number of flower, ranging from 0 to 1. The relative reproductive success of an individual was defined as its fruit set divided by the mean population fruit set. Morphological traits were scaled by population mean and standard deviation (z-scores, Lynch & Walsh, 1998). To compare selection differentials over different years, we computed bootstrap bias-corrected confidence intervals (CIs: Maad & Alexandersson, 2004). Non-overlapping CIs indicated significantly different selection differentials, CIs including 0 were deemed be non-significant.

To estimate selection gradients, following Lande & Arnold (1983), we performed a single multiple linear regression using relative fitness as dependent variable and standardized traits as predictors.

RESULTS

All floral traits were moderately positively correlated with the exception of carotenoid content in tepals and labellum, which were negatively correlated with other phenotypic traits (Table 1). In both species, several floral traits showed significant differences across different years (Fig. 1, Fig. 2).

In both investigated species, CVs were extremely high for all floral traits indicating a high variability of floral traits with a slight variation across years. Average CV was 0.35 in *Orchis mascula* and 0.42 in *Orchis pauciflora* (See Supporting information).

Page: 15

Number: 1 Author: Subject: Sticky Note Date: 5/31/17, 4:39:03 PM -04'00'

linear regressions assume that the data are normally distributed. Are they? If they are not normal and cannot be successfully transformed, then there are other approaches where you can use variables with beta, poisson, or binomial distributions

Data from Scopece *et al.* (2007) showed that, in both species, the ratio between hand-pollinated flowers and fruits developed was very high (1 for *Orchis mascula* and 0.92 for *O. pauciflora*), in contrast with fruit formation in open-pollinated individuals, which was low (0.11 in *O. mascula* and 0.10 in *O. pauciflora*). Thus, both species were severely pollen-limited (PL for *O. mascula* = 0.89; PL for *O. pauciflora* = 0.87).

All of the investigated floral traits in both species showed some amount of selection across years (Fig. 1, Fig. 2). A comparison of the selection differentials in three consecutive years revealed significant differences for 7 traits in both *O. mascula* (Fig. 1) and *O. pauciflora* (Fig. 2). Specifically, in *Orchis mascula*, selection differentials were significantly different for 6 out of 9 traits between 2002 and 2003, 3 out of 9 between 2003 and 2004, and 3 out of 9 between 2002 and 2004 (Fig. 1). In *Orchis pauciflora*, selection differentials were significantly different for 2 out of 9 traits between 2002 and 2003, 6 out of 9 between 2003 and 2004, and 2 out of 9 between 2002 and 2004 (Fig. 2).

Across the three years, the selection differential patterns were generally consistent with floral trait variation (i.e., decrease when floral traits increase). In *O. mascula*, the patterns were consistent in 7 out of 9 traits. In *O. pauciflora*, the patterns were consistent in 6 out of 9 traits.

We also found changes in the direction of selection (i.e., signs of selection differential) in 3 out of 9 floral traits in *O. mascula* (Fig. 1) and in 3 out of 9 floral traits in *O. pauciflora* (Fig. 2). Selection gradients for each investigated population are reported in Table 2. Results show only few marginally significant values indicating weak direct selection on floral traits.

Number: 1 Author: Subject: Sticky Note Date: 5/29/17, 11:33:08 PM -04'00'

line 246: Do you mean that floral trait variation increases?

This page contains no comments

DISCUSSION

The quantification and interpretation of the direction, strength and causes of natural selection have been at the centre of scientific debate since the formulation of Darwin's theory (Darwin, 1859). Among the main aims of studies on selection is the capture of snapshots and milestones in the process of phenotypic trait evolution mediated by natural selection. Thus far, many studies have shown selection in action on different animal or plant organisms (Kingsolver et al., 2001; Hereford et al., 2004), generating the idea that directional selection is widespread in natural populations. This idea is confirmed by the general stability of phenotypic traits observed in rewarding plant species, but it apparently conflicts with the elevated polymorphism observed in deceptive species (Ackerman et al., 2011). In this study, we estimated selection differentials and gradients for nine floral traits in two Mediterranean food-deceptive orchid species, *Orchis mascula* and *O. pauciflora* over three consecutive years. Overall, we found some degree of selection on all of the investigated traits but a strong variation in direction and intensity over different years (Fig. 1, Fig. 2). As expected in deceptive orchids (Tremblay et al., 2005), we found that the two investigated species showed high levels of pollinator limitation. Although pollen limitation is not predictive of pollinator-mediated selection, in severely pollen-limited species the strength of selection is mainly due to the action of pollinators (Sletvold et al., 2010; Sletvold & Agren, 2014). Despite increasing evidence showing pollinator-mediated selection in Mediterranean food-deceptive orchids (Johnson & Steiner, 1997; O'Connell & Johnston, 1998; Johnson & Nilsson, 1999; Sun et al., 2009; Sletvold et al., 2010; Zitari et al., 2012; Sletvold et al., 2016), our results showed a weak and variable selection on floral traits when analysed

This page contains no comments

over different, consecutive seasons suggesting the absence of constant directional selection on these species. This can potentially be responsible for the elevated phenotypic polymorphism that we directly assessed in the investigated species through the calculation of coefficients of variation for morphological traits that were on average higher than those reported in previous literature surveys on deceptive orchid species (i.e. 0.35 in *O. mascula* and 0.42 in *O. pauciflora versus* an average of 15.2% in the deceptive species and of 11.1% for the rewarding species reported in Ackerman et al., 2011). Indeed, we found that selection differentials significantly varied at least once in direction or strength in 7 out of 9 traits in both *O. mascula* and *O. pauciflora* over different years (Fig. 1, Fig. 2) with a total of 12 changes out of 27 comparisons in *O. mascula* (Fig. 1) and 10 changes out of 27 in *O. pauciflora* (Fig. 2). In both investigated species, most of the studied floral traits were positively correlated (Table 1), which could in principle mask the action of natural selection in the selection differential analysis (Lande & Arnold, 1983). However, our analysis of selection gradients showed only a few marginally significant results, thus confirming the weak selection observed in selection differentials (see Table 2).

Floral traits were not constant over different years and most of these traits showed significant differences in different reproductive seasons (Fig. 1, Fig. 2). In several cases, selection differentials were consistent with this variation; e.g., in *O. mascula*, plant height was under stronger selection when plants were less tall (Fig. 1), thus suggesting a weak directional selection over the years. However, for other traits, selection differentials were inconsistent with this variation (Fig. 1, Fig. 2), which suggests that

This page contains no comments

fluctuations of natural selection for these traits are not an artefact but rather reflect the activities of different selective agents in different years.

Significant differences among floral traits in different years, within the same population, may be explained as a consequence of phenotypic plasticity. Indeed, in a variable environment, a single generalist genotype can potentially express a wide range of random phenotypes or show different responses to environmental cues via phenotypic plasticity (Kawecki & Ebert, 2004; Hill & Mulder, 201 In our study, this can be accentuated by the extreme climatic conditions of the 2003 heat wave (Beniston, 2004). Interestingly, in 2003, measurements showed a quantitative reduction of most floral traits (Fig. 1, Fig. 2). However, despite the contribution of plasticity on the phenotypic expression of the investigated floral traits, their heritability was verified by a comparison of phenotypic expression of traits in hybrid zones (Scopece et al., in prep.). Fluctuating selection has been proposed to be common in natural populations (Siepielski et al., 2009; Kimball et al., 2012), but its potential role is still debated particularly due to the dearth of extensive multi-year studi In our study, with a multi-year dataset, we showed that the change in the direction and intensity of selection on the same floral traits could determine a continuous but fluctuating pressure that favours different individuals in different years. Within the same population, this can potentially result in the maintenance of variable phenotypes. Indeed, although changes in selective pressures in annual plant species may generate a displacement of phenotypic traits, in perennial long-lived plants such as orchids, the individual's reproductive success is the result of its performance during its lifetime and thus only selection pressures that are constant over time can generate displacement or stabilisation of the distribution of

Page: 20

Number: 1 Author: Subject: Sticky Note Date: 5/28/17, 7:55:09 PM -04'00'

Indeed. See Morales et al. 2010. Morphological flexibility across an environmental gradient in the epiphytic orchid, Tolumnia variegata: complicating patterns of fitness. Bot J Linn Soc 163: 431-446. They show phenotypic plasticity and annual variatin in environmental conditions can muddy the selection trajectories.

Number: 2 Author: Subject: Sticky Note Date: 5/28/17, 7:41:31 PM -04'00'

Again, the Tremblay et al. 2010 paper is applicable here.

phenotypic traits. In contrast, fluctuating pressures are more likely to explain the phenotypic polymorphism observed in the natural populations of deceptive orchids. The main source of this fluctuating selection is most likely the action of pollinators. In the nectar-rewarding orchid *Gymnadenia conopsea* spatio-temporal variation in interactions with pollinators contributes to among-years and among-populations variation in selection on floral traits but that several traits are also likely to be subject to different selective agents (Sletvold & Ågren, 2010; Chapurlat et al., 2015). In our study, we focused on severely pollen-limited food-deceptive species in which pollinators are likely to be the main selective agents (Tremblay et al., 2005; Sletvold et al., 2010; Sletvold & Ågren, 2014). Furthermore, direct estimation based on the comparison of reproductive performance between open-pollinated and hand-pollinated plants confirmed elevated levels of pollinator limitation for the two investigated species (PL for *O. mascula* = 0.89; PL for *O. pauciflora* = 0.87). Pollinator-mediated selection is a complex process that can be affected by a high number of environmental variables. For example, pollinators with different tongue lengths may exert different selection pressures that positively select flowers with shorter or longer nectar spurs (Johnson & Steiner, 1997). However, pollinator community varies over the flowering season and in different years depending on climatic differences that can alter both the phenology of plants and emergence of pollinators. Moreover, for similar reasons, the surrounding plant community may change in different years, thereby influencing local pollinator preference and abundance. The change in pollinators or surrounding plant community composition is particularly crucial for generalist deceptive species such as *O. mascula* and *O. pauciflora*, which attract a wide range of available pollinator species, and is the

Number: 1 Author: Subject: Sticky Note Date: 5/31/17, 4:21:37 PM -04'00'

cite some references for the last two sentences, such as Schemske and Horvitz papers on year-to-year variation in pollinator pool in *Calathea* -- e.g., Schemske DW, Horvitz CC. 1989. Temporal variation in selection on a flora character. Evolution 43:461-465. also Herrera 1988 Biol J Linn Soc, and some more recent literature most likely source of the observed changes in selection differentials. Changes in pollinator-mediated selection have been widely documented in plant species, including orchids, and have been attributed to many environmental variables (e.g., Caruso et al., 2003; Conner et al., 2003; Gòmez et al., 2003; Toräng et al., 2008; Sletvold & Ågren, 2010). However, identifying the source of variation in selection differentials can only be achieved via detailed community ecological studies at the local scale. For instance, similar attempts have previously shown that vegetation height affects the strength of pollinator-mediated selection in the food-deceptive orchid *Dactyloriza lapponica* (Sletvold et al., 2013), thus suggesting that variation in selection also occurs within the same reproductive season at small geographic scales.

Future research avenues should address the basis of the elevated phenotypic polymorphism of food-deceptive species and confirm whether similar variation occurs even at a fine geographic scale. Such studies should include fine scale community ecological investigations, as for instance temporal and local variation in pollinator community, that aim to disentangle the factors affecting variation. Simultaneously, it would also be important to conduct more studies on nectar-rewarding orchids to test for the opposite pattern, i.e., lower variability of selection pressures in space and time.

ACKNOWLEDGEMENTS

Authors thank A.M. Nardella and S. Impagliazzo for help with field-work and the Cilento and Vallo di Diano National Park for permissions and logistic support. The authors also thank Elodie Chapurlat who revised a previous version of this manuscript. This research

This page contains no comments

was carried out in the frame of Programme STAR, financially supported by UniNA and Compagnia di San Paolo.

REFERENCES

Aceto S, Caputo P, Cozzolino S, Gaudio L, Moretti A. 1999. Phylogeny and evolution of *Orchis* and allied genera based on ITS DNA variation: morphological gaps and molecular continuity. *Molecular Phylogenetics and Evolution* **13**:67–76.

Ackerman JD, Cuevas AA, Hof D. 2011. Are deception-pollinated species more variable than those offering a reward? *Plant Systematics and Evolution* **293**:91–99.

Ackerman JD, Galarza-Pérez M. 1991. Patterns and maintenance of extraordinary variation in the Caribbean orchid, *Tolumnia* (*Oncidium*) *variegata*. *Systematics Botany* **16**:182–194.

Bateman RM, Rudall PJ. 2006. Evolutionary and morphometric implications of morphological variation among flowers within an inflorescence: a case-study using European orchids. *Annals of Botany* **98**:975–993.

Beniston M. 2004. The 2003 heat wave in Europe: A shape of things to come? An analysis based on Swiss climatological data and model simulations. *Geophysical Research Letters* **31**:1–4.

Benitez-Vieyra S, Ordano M, Fornoni J, Boege K, Domínguez CA. 2010. Selection on signal–reward correlation: limits and opportunities to the evolution of deceit in *Turnera ulmifolia* L. *Journal of Evolutionary Biology* **23**:2760–2767.

This page contains no comments

Benitez-Vieyra S, Glinos E, Medina AM, Cocucci AA. 2012. Temporal variation in the selection on floral traits in *Cyclopogon elatus* (Orchidaceae). *Evolutionary Ecology* **26**:1451–1468.

Bradshaw HD, Otto KG, Frewen BE, McKay JK, Schemske DW. 1998. Quantitative trait loci affecting differences in floral morphology between two species of monkeyflower (*Mimulus*). *Genetics* **149**:367–382.

Brodie ED, Moore AJ, Janzen FJ. 1995. Visualizing and quantifying natural selection. *Trends in Ecology and Evolution* **10**:313–318.

Caruso CM, Peterson SB, Ridley CE. 2003. Natural selection on floral traits of *Lobelia* (Lobeliaceae): spatial and temporal variation. *American Journal of Botany* **90**:1333–1340.

Chapurlat E, Ågren J, Sletvold N. 2015. Spatial variation in pollinator-mediated selection on phenology, floral display and spur length in the orchid *Gymnadenia conopsea*. *New Phytologist* **208**:1264–1275.

Conner JK, Rice AM, Stewart C, Morgan MT. 2003. Patterns and mechanisms of selection on a family-diagnostic trait: evidence from experimental manipulation and lifetime fitness selection gradients. *Evolution* **57**:480–486.

Cozzolino S, Nardella AM, Impagliazzo S, Widmer A, Lexer C. 2006. Hybridization and conservation of Mediterranean orchids: should we protect the orchid hybrids or the orchid hybrid zones? *Biological Conservation* **129**:14–23.

Dafni A. 1984. Mimicry and deception in pollination*. Annual Review of Ecology and Systematics* **15**:259–278.

Darwin CR. 1859. On the origin of species by means of natural selection, or the

This page contains no comments

preservation of favoured races in the struggle for life. London: John Murray.

Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD. 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology and Systematics* **35**:375–403.

Gigord LDB, Macnair MR, Smithson A. 2001. Negative frequency-dependent selection maintains a dramatic flower color polymorphism in the rewardless orchid *Dactylorhiza sambucina* (L.) Soò. *Proceedings of the National Academy of Sciences USA* **98**:6253–6255.

Gòmez JM. 2003. Herbivory reduces the strength of pollinator-mediated selection in the Mediterranean herb *Erysimum mediohispanicum*: consequences for plant specialization. *American Naturalist* **162**:242–256.

Grant BR, Grant PR. 1989. Natural selection in a population of Darwin’s finches. *American Naturalist* **133**:377–393.

Gumbert A. 2000. Color choices by bumble bees (*Bombus terrestris*): innate preferences and generalization after learning. *Behavioral Ecology and Sociobiology* **48**:36–43.

Harder LD, Johnson SD. 2009. Darwin's beautiful contrivances: evolutionary and functional evidence for floral adaptation. *New Phytologist* **183**:530–545.

Heinrich B. 1975. Bee flowers: a hypothesis on flower variety and blooming times. *Evolution* **29**:325–334.

Hereford J, Hansen TF, Houle D. 2004. Comparing strengths of directional selection: how strong is strong? *Evolution* **58**:2133–2143.

Hill WG, Mulder HA. 2010. Genetic analysis of environmental variation. *Genetics*

This page contains no comments

Research **92**:381–395.

Holderegger R, Kamm U, Gugerli F. 2006. Adaptive vs. neutral genetic diversity: implications for landscape genetics. *Landscape Ecology* **21**:797– 807.

Jersàkovà J, Johnson SD, Kindlman P. 2006. Mechanisms and evolution of deceptive pollination in orchids. *Biological Reviews* **81**:219–235.

Johnson SD, Steiner KE. 1997. Long-tongued fly pollination and evolution of floral spur length in the *Disa draconis* complex (Orchidaceae). *Evolution* **51**:45–53.

Johnson SD, Nilsson LA. 1999. Pollen carryover, geitonogamy, and the evolution of deceptive pollination system in orchids. *Ecology* **80**:2607–2619.

Juillet N, Scopece G. 2010. Does floral trait variability enhance reproductive success in deceptive orchids? *Perspectives in Plant Ecology Evolution and Systematics* **12**:317–322.

Kawecki TJ, Ebert D. 2004. Conceptual issues in local adaptation. *Ecology Letters* **7**, 1225–1241.

Kimball S, Gremer JR, Angert AL, Huxman TE, Venable DL. 2012. Fitness and physiology in a variable environment. *Oecologia* **169**:319–329.

Kingsolver JG, Hoekstra HE, Hoekstra JM, Berrigan D, Vignieri SN, Hill CE, Hoang A, Gibert P, Beerli P. 2001. The strength of phenotypic selection in natural populations. *American Naturalist* **157**:245–261.

Knapp EE, Rice KJ. 1998. Comparison of isozymes and quantitative traits for evaluating patterns of genetic variation in purple needlegrass (*Nassella pulchra*). *Conservation Biology*, **12**:1031–1041.

Lande R, Arnold SJ. 1983. The measurement of selection on correlated characters.

This page contains no comments

Evolution **37**:1210–1226.

Lawton-Rauh A. 2008. Demographic processes shaping genetic variation. *Current Opinion in Plant Biology* **11**:103–109.

Lynch M, Walsh B. 1998. Genetics and Analysis of Quantitative Traits. Sunderland: Sinauer Associate.

Maad J. 2000. Phenotypic selection in hawkmoth-pollinated *Platanthera bifolia*: targets and fitness surfaces. *Evolution* **54**:112–123.

Maad J, Alexandersson R. 2004. Variable selection in *Platanthera bifolia* (Orchidaceae): phenotypic selection differed between sex functions in a drought year. *Journal of Evolutionary Biology* **17**:642–650.

Moya S, Ackerman JD. 1993. Variation in the floral fragrance of *Epidendrum ciliare* (Orchidaceae). *Nordic Journal of Botany* **13:**41–47.

Nilsson LA. 1980. The pollination ecology of *Dactylorhiza sambucina* (Orchidaceae). *Botaniska Notiser* **133**, 367–385.

Nilsson LA. 2008. Anthecology of *Orchis mascula* (Orchidaceae). *Nordic Journal of Botany* **3**:157–179.

O’Connell LM, Johnston MO. 1998. Male and female pollination success in a deceptive orchid: a selection study. *Ecology* **79**:1246–1260.

Salzmann CC, Nardella AM, Cozzolino S, Schiestl FP. 2007. Variability in floral scent in rewarding and deceptive orchids: the signature of pollinator-imposed selection? *Annals of Botany* **100**:757–765.

Sandring S, Riihimäki MA, Savolainen O, Ågren J. 2007. Selection on flowering time and floral display in an alpine and a lowland population of *Arabidopsis lyrata*. *Journal*

This page contains no comments

of Evolutionary Biology **20**:558–567.

Schiestl FP. 2005. On the success of a swindle: pollination by deception in orchids. *Naturwissenschaften* **92**:255–264.

Schiestl FP, Johnson SD. 2013. Pollinator-mediated evolution of floral signals. *Trends in Ecology and Evolution* **28**:307–315.

Scopece G, Musacchio A, Widmer A, Cozzolino S. 2007. Patterns of reproductive isolation in Mediterranean deceptive orchids. *Evolution* **61**:2623–2642.

Siepielski AM, Di Battista JD, Carlson SM. 2009. It's about time: the temporal dynamics of phenotypic selection in the wild. *Ecology Letters* **12**:1261–1276.

Sletvold N, Grindeland JM, Ågren J. 2010. Pollinator-mediated selection on floral traits and flowering phenology in the deceptive orchid *Dactylorhiza lapponica*. *New Phytologist* **188**:385–392.

Sletvold N, Ågren J. 2010. Pollinator-mediated selection on floral display and spur length in the orchid *Gymnadenia conopsea*. *International Journal of Plant Sciences*, **171**:999–1009.

Sletvold N, Grindeland JM, Ågren J. 2013. Vegetation context influences the strength and targets of pollinator-mediated selection in a deceptive orchid. *Ecology* **94**:1236–42.

Sletvold N, Ågren J. 2014. There is more to pollinator-mediated selection than pollen limitation. *Evolution* **68**:1907–1918.

Sletvold N, Trunschke J, Smit M, Verbeek J, Ågren J. 2016. Strong pollinator-mediated selection for increased flower brightness and contrast in a deceptive orchid. *Evolution* **70**:716–724.

This page contains no comments

Smithson A, Gigord LDB. 2003. The evolution of empty flowers revisited. *American Naturalist*, **161**:537–552.

Stejskal K, Streinzer M, Dyer A, Paulus HF, Spaethe J 2015. Functional significance of labellum pattern variation in a sexually deceptive orchid (*Ophrys heldreichii*): Evidence of individual signature learning effects. *Plos One* **10**:e0142971.

Sun H-Q, Cheng J, Zhang F-M, Luo, Y-B, Ge S. 2009. Reproductive success of non-rewarding *Cypripedium japonicum* benefits from low spatial dispersion pattern and asynchronous flowering. *Annals of Botany* **103**:1227–1237.

Sundermann H. 1980. Europäische und mediterrane Orchideen. Hildesheim: Brücke Verlag Kurt Schmersow.

Toräng P, Ehrlén J, Ågren J. 2008. Mutualists and antagonists mediate frequency-dependent selection on floral display. *Ecology* **89**:1564–1572.

Tremblay RL, Ackerman JD, Zimmerman JK, Calvo RN. 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.

Valterovà I, Kunze J, Gumbert A, Luxovà A, Liblikas I, Kalinovà B, Borg-Karlson A-B. 2007. Male bumble bee pheromonal components in the scent of deceit pollinated orchids; unrecognized pollinator cues? *Arthropod–Plant Interactions* **1**:137–145.

Van Der Cingel NA. 1995. An atlas of orchids pollination–European orchids. Rotterdam, Netherlands: Balkema.

Waser NM. 1986. Flower constancy: definition, cause, and measurement. *American Naturalist* **127**:593–603.

This page contains no comments

Zitari A, Scopece G, Helal AN, Widmer A, Cozzolino S. 2012. Is floral divergence sufficient to maintain species boundaries upon secondary contact in Mediterranean food-deceptive orchids? *Heredity* **108**:219–228.

Figure legends

Figure 1 Morphological traits (white bars) and selection differentials (grey bars) in *Orchis mascula*. Different letters indicate significant differences.

Figure 2 Morphological traits (white bars) and selection differentials (grey bars) in *O. pauciflora*. Different letters indicate significant differences.

Supplementary figure 1 Scanned digital images of A) *O. pauciflora* and B) *O. mascula* floral parts. From left to right: spur, labellum.

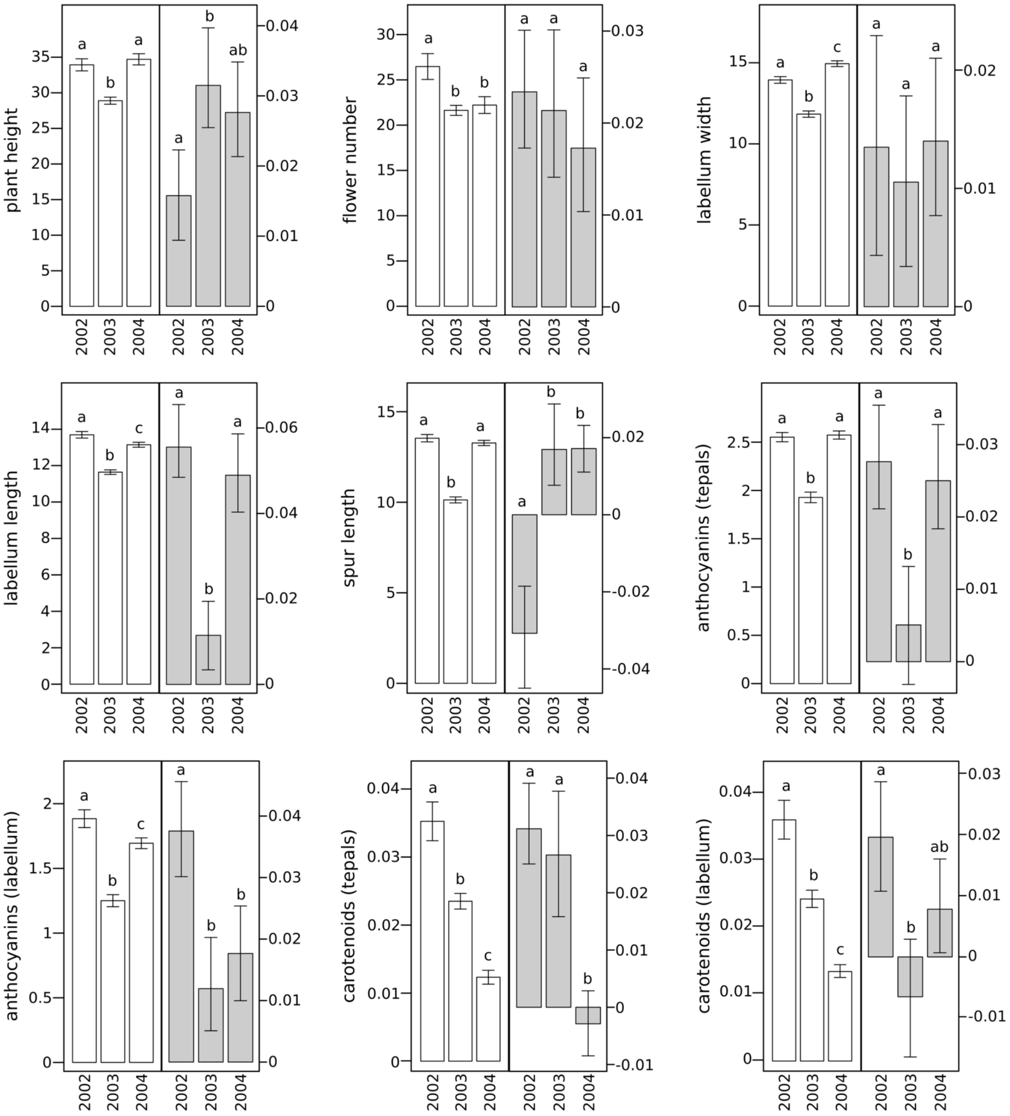
This page contains no comments

Figure 1(on next page)

Morphological traits and selection differentials in Orchis mascula

Morphological traits (white bars) and selection differentials (grey bars) in *Orchis mascula*. Different letters indicate significant differences.

This page contains no comments


plant height
0
5
10
15
20
25
30
35
0
0.01
0.02
0.03
0.04
a
b
a
a
b
ab
flower number
0
5
10
15
20
25
30
0
0.01
0.02
0.03
a
b
b
a
a
a
labellum width
0
5
10
15
0
0.01
0.02
a
b
c
a
a
a
labellum length
0
2
4
6
8
10
12
14
0
0.02
0.04
0.06
a
b
c
a
b
a
spur length
0
5
10
15
-0.04
-0.02
0
0.02
a
b
a
a
b
b
anthocyanins (tepals)
0
0.5
1
1.5
2
2.5
0
0.01
0.02
0.03
a
b
a
a
b
a
anthocyanins (labellum)
0
0.5
1
1.5
2
0
0.01
0.02
0.03
0.04
a
b
c
a
b
b
carotenoids (tepals)
0
0.01
0.02
0.03
0.04
-0.01
0
0.01
0.02
0.03
0.04
a
b
c
a
a
b
carotenoids (labellum)
0
0.01
0.02
0.03
0.04
-0.01
0
0.01
0.02
0.03
a
b
c
a
b
ab
2002
2003
2004
2002
2003
2004

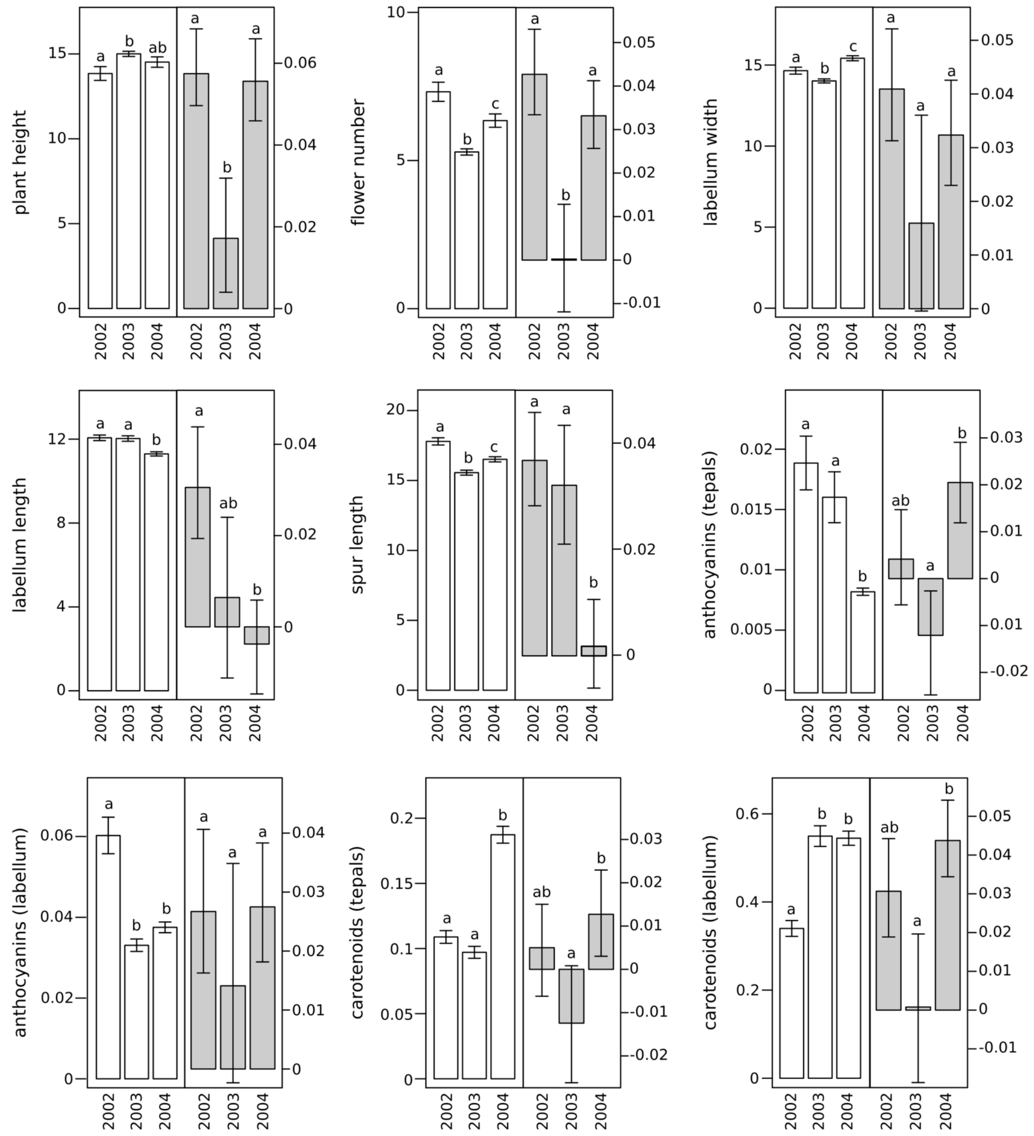
This page contains no comments

Figure 2(on next page)

Morphological traits and selection differentials in O. pauciflora.

Morphological traits (white bars) and selection differentials (grey bars) in *O. pauciflora*. Different letters indicate significant differences.

This page contains no comments


plant height
flower number
labellum width
labellum length
spur length
anthocyanins (tepals)
anthocyanins (labellum)
carotenoids (tepals)
carotenoids (labellum)
2002
2003
2004
a
b
ab
c

This page contains no comments

Table 1(on next page)

Phenotypic Correlations

Phenotypic Correlations (Spearman’s rank) among morphological traits in *Orchis mascula* (above diagonal) and *O. pauciflora* (below diagonal). All plants from the three years were pooled together.

This page contains no comments

Table 1 Phenotypic Correlations (Spearman’s rank) among morphological traits in *Orchis mascula* (above diagonal) and *O. pauciflora* (below diagonal). All plants from the three years were pooled together.

	Plant heigth	Flower number	Labellum width	Labellum length	Spur length	Anthocyanins content in tepals	Anthocyanins content in labellum	Carotenoid content in tepals	Carotenoid content in labellum
Plant heigth		0.76***	0.47***	0.48***	0.44***	0.29***	0.25***	0.07	-0.07
Flower number	0.51***		0.25***	0.30***	0.24***	0.15**	0.14**	0.15**	0.06
Lebellum width	0.32***	0.28***		0.71***	0.69***	0.39***	0.48***	-0.16**	-0.09
Labellum length	0.26***	0.22***	0.49***		0.63***	0.40***	0.50***	0.01	0.06
Spur length	0.22***	0.10*	0.44***	0.14**		0.47***	0.50***	-0.05	-0.05
Anthocyanins content in tepals	0.16**	0.19***	0.08	0.22***	0.01		0.66***	-0.01	-0.08
Anthocyanins content in labellum	0.18***	0.42***	0.31***	0.24***	0.08	0.45		-0.01	0.01
Carotenoid content in tepals	-0.09*	0.21***	0.19***	-0.03	-0.05	-0.05	0.18***		0.43***
Carotenoid content in labellum	0.24***	0.19***	0.30***	0.10*	0.00	0.18***	0.25***	0.32***	

This page contains no comments

***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

This page contains no comments

Table 2(on next page)

Selection gradients

Selection gradients (ß) and their significance (p) calculated according to Lande and Arnold (1983).

This page contains no comments

Table 2 Selection gradients (ß) and their significance (p) calculated according to Lande and Arnold (1983)

	Flower number		Plant height		Labellum width		Labellum length		Spur length		Anthocyanins content in tepals		Anthocyanins content in labellum		Carotenoid content in tepals		Carotenoid content in labellum	
	ß	p	ß	p	ß	p	ß	p	ß	p	ß	p	ß	p	ß	p	ß	p
O. mascula 2002	0.440	0.020	-0.203	0.269	-0.231	0.140	0.462	0.002	-0.217	0.065	0.172	0.212	0.084	0.518	0.066	0.680	0.149	0,369
O. pauciflora 2002	0.030	0.853	0.281	0.138	-0.040	0.817	0.005	0.970	0.095	0.476	-0.038	0.734	0.184	0.118	0.030	0.793	0.005	0,969
O. mascula 2003	-0.119	0.389	0.173	0.248	0.018	0.865	0.070	0.545	0.065	0.590	-0.033	0.759	0.028	0.791	0.245	0.005	-0.062	0,477
O. pauciflora 2003	-0.132	0.157	0.135	0.152	0.017	0.850	0.101	0.198	0.041	0.646	-0.080	0.330	0.095	0.291	-0.016	0.841	-0.018	0,847
O. mascula 2004	-0.063	0.664	0.196	0.223	-0.258	0.032	0.538	0.000	-0.076	0.453	0.150	0.155	-0.102	0.359	-0.119	0.195	0.021	0,810
O. pauciflora 2004	-0.122	0.316	0.414	0.002	0.295	0.030	-0.411	0.001	-0.101	0.321	0.039	0.695	0.030	0.771	-0.054	0.586	0.187	0,087

Page: 39

Number: 1 Author: Subject: Sticky Note Date: 5/28/17, 9:07:10 PM -04'00'

boldface the significant selection gradients -- it would make scanning the table easier.