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EVIDENCE OF ARTIFICIAL SELECTION: ARE ORCHIDS IN CULTIVATION AN EFFECTIVE *EX SITU* CONSERVATION STRATEGY?

Raymond L. Tremblay[1,2], Paola A. Alicea-Roman[1], Abimelys Anaya-Reyes[1], Sean Duclerc-Rodas[1] & Ivanellys Medina-Tirado[1]

[1]Department of Biology, University of Puerto Rico, Humacao campus, José E. Aguiar Aramburu, Carr. 908, Km 1.2, Humacao, Puerto Rico, PR, 00791.

[2]Author for correspondence: raymond.tremblay@upr.edu

Abstract. Artificial selection is the process by which humans change morphological and genetic characteristics of species through selection of "favored" characters. Although the gray literature suggests the process is occurring in orchids held in *ex situ* collections, there is limited evidence of this in the scientific literature. There is a perspective among growers that species (not hybrids) held in *ex situ* collections are potential sources of material for use in *in situ* re-establishment, however, this assumes that there has not been any artificial selection for morphological characters, or functional traits while grown and propagated *ex situ*. Here we evaluate if plants grown in *ex situ* collections show changes in morphological characters across time and if the range of character size is within the range from *in situ* populations. We evaluated plants from the American Orchid Society database from nine genera and 54 species. We noted that 35% of characters evaluated had evidence of significant change across time. Moreover, for most species in *ex situ* the evaluated characters were frequently (95%) outside the range of plants of natural populations based on species descriptions. If variation in size of *ex situ* collections as compared to *in situ* plants is genetically based, it is possible that these would be functionally maladaptive if re-introduced to their natural environment. Protocols for *ex situ* conservation programs need to focus on the morphological, biochemical, and ecological interactions and genetic diversity that would render the re-introduction of *ex situ* to their natural environment to maximize the likelihood of effective re-establishment. Consequently, species which are awarded recognition at orchid shows may potentially be inappropriate for plants within an *in situ* reintroduction conservation program.

Resumen. La selección artificial es el proceso por el cual los humanos cambian las características morfológicas y genéticas de las especies a través de la selección de caracteres "favorecidos". Aunque la literatura gris sugiere que el proceso está ocurriendo en orquídeas mantenidas en colecciones *ex situ*, existe evidencia limitada de esto en la literatura científica. Esta presente una perspectiva común entre los cultivadores de que las especies (no los híbridos) que se mantienen en las colecciones *ex situ* son fuentes potenciales de material para usar en el restablecimiento *in situ*, sin embargo, esto supone que no ha habido ninguna selección artificial de caracteres morfológicos, o rasgos funcionales mientras crecían y se propagaban *ex situ*. Aquí evaluamos si las plantas cultivadas en colecciones *ex situ* muestran cambios en las características morfológicas a lo largo del tiempo y si el rango de tamaño de los fenotipos está dentro del rango de las poblaciones *in situ*. Evaluamos plantas de la base de datos de la *American Orchid Society* de nueve géneros y 54 especies. Notamos que el 35% de los caracteres evaluados tenían evidencia de cambios significativos a lo largo del tiempo. Además, para la mayoría de las características evaluadas de las especies *ex situ* estaban frecuentemente (95%) fuera del rango de plantas de poblaciones naturales según las descripciones de las especies. Si la variación en el tamaño de las plantas en colecciones *ex situ* en comparación con las plantas *in situ* tiene una base genética, es posible que estas sean funcionalmente inadaptadas si se reintroducen en su entorno natural. Los protocolos para los programas de conservación *ex situ* deben centrarse en las interacciones ecológicas incluyendo la variación morfológica, bioquímicas y la diversidad genética que harían que la reintroducción de plantas *ex situ* a su entorno natural maximizara la probabilidad del restablecimiento efectivo. Por consecuencia, las especies que reciben reconocimiento en las exposiciones de orquídeas pueden ser potencialmente inapropiadas dentro de un programa de conservación de reintroducción *in situ*.

ORCID of the Authors: RLT , PAAR , AAR , IMT
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Introduction. Throughout history humans have modified animal and plant characteristics through artificial selection, this process started early when humans domesticated wild animals and plants (Conner 2003) from about 13,000 years ago (Diamond 2002). Evidence for artificial selection of plant characteristics is numerous, and the history leading to artificial selection in many plant species is well known (Cantarel *et al.* 2021, Casas 2007, Fujino 2021). For example, humans have artificially selected floral characteristics of many species (Callahan 2005, Van Tassel 2010), often through hybridization processes (Abreu *et al.* 2009, Datta 2021, Marasek-Ciolakowska *et al*. 2021, Seehausen 2004). Contrary to the development of floral cultivars, which attempts to "discover" new morphological variants, *ex situ* conservation strategies have the objective of preserving the genetic and morphological diversity observed of in *in situ* populations (Basey *et al.* 2015, Cibrian-Jaramillo *et al.* 2013, Guerrant Jr. *et al.* 2014, Havens *et al.* 2004, 2006). The literature on conservation biology and *ex situ* conservation has explored many aspects for plant species conservation including orchids (Swarts & Dixon 2017). Sample of the diversity of topics that have been explored includes the philosophical and ethical aspects (Chan *et al*. 2007, Evans 2021), genetic consequences of varying models of e*x situ* conservation of germplasms (Engels & Ebert 2021), the effective use of gene banks (Seaton 2007, Wei & Jiang 2021), the effectiveness of cryopreservation (Das *et al.* 2021, Kaur 2019, Swarts & Dixon 2009). What is particularly important is developing a better understanding of the complexity of effective strategies which would lead to successful reintroduction.

A common comment of orchid enthusiasts has been that plant species found in private, public gardens and plant growers' collections are sources of plants for *ex situ* conservation (Shirey *et al.* 2013). Multiple postulations are encompassed within this perspective. First, a common one is that because of deforestation and environmental impact by humans, at least some individuals of threatened species are conserved (Hinsley *et al.* 2018). Thus, an *ex situ* assemblages of plants of a species are a repository of genetic and morphological diversity. A second postulation that plant growers often express is that survivorship of plants in these controlled environments are higher than that in the natural habitat; thus, *ex situ* conservation is an effective strategy. Finally, a third postulation is that the number of individuals in a private or plant grower's environment can be increased much faster than in the natural environment. There is no doubt that many examples support each of these comments thus warranting in part being considered "*ex situ* conservation". For an introduction to guidelines to some of the variables which should be considered for *ex situ* conservation see Engels & Ebert (2021) and for quantitative approaches see Ferson & Burgman (2000).

Perusing the literature on effective protocols for *ex situ* conservation where the objective is to conserve the genetic and morphological variation of a species are multi-faceted and complex and require a multidisciplinary team and an *a priori* protocol for maximizing the diversity of genetic and morphological variation across populations of the species of interest (Abeli *et al.* 2019, Engels *et al.* 2021, Griffith *et al.* 2017, Li & Pritchard 2009, Phillips *et al.* 2020, Volis & Blecher 2010, Volis *et al.* 2009, and references within). It is clear from the present knowledge of the field that there are many possible drawbacks if a protocol fails to be comprehensive in capturing the species diversity (genetics and morphological) and maintaining it while in an *ex situ* collections (Curio 1996).

It is further assumed that the artificial environment or management practice while in *ex situ,* is not selecting for specific characteristics and if these were relocated to an adequate natural environment these plants would flourish. In other words, during the period of *ex situ* conservation it is assumed that there is no artificial selection (change in genetic or morphological diversity) that would make these individuals less apt for a successful reintroduction (Vitt & Havens 2004). When the process of *ex situ* conservation is applied scientifically, the collectors are likely to try to maximize the genetic diversity and morphological quantitative variation found in its natural environment that may ultimately be adaptive in the re-introduction phase (Fiani *et al*. 2021). For example, seed banks regeneration guidelines aim to minimize the effect of genotype selection (Phartyal *et al*. 2002) and the impact of inconsistencies in the protocol have been studied (Hay *et al*. 2021). However, during the *ex situ* conservation process, this is likely to be a non-random process (mainly if this occurs across multiple generations). Growers often select for more robust individuals assuming that these would have a higher fitness (for example, more flowers, longer life span, reduce mortality, resistance to pathogens, etc.). More "robust" plants in controlled environments (i.e. a greenhouse, botanical gardens) may not be adaptive in the natural environment. How common this process has not been quantified, and an attempt to identify peer-reviewed articles on "artificial selection" and "orchids" using Google Scholar has resulted in very few hits (Li *et al.* 2021) let alone evaluating if artificial selection while in *ex situ* collections is occurring and/or maladaptive for effective re-introduction. Su *et al.* (2018) showed change in morphological characters in lineages of *Cymbidium sinense* (Jacks. ex Andrews) Willd. which have been in cultivation for centuries and its correlation with transcriptome analysis of MADS genes. Character selection with economical use such as vanillin and other compounds in *Vanilla* Mill. cultivars have also been studied (Salazar-Rojas *et al.* 2012). Li *et al*. (2021) discussed technological advances and methods for more effective artificial selection in orchids and review the present knowledge of heritable characters in orchids. Although the gray literature (non-peer reviewed publications) is inundated with examples of selection for varying characters of species in cultivation (for examples see specialized Orchid popular journals "Orchids Magazine", "Orchid Digest", "Orchid Review"), we do not have a wide and scientific perspective of the effect of cultivation on the frequency of artificial selection on one of the most charismatic plant family.

In an *ex situ* environment, plants are grown in a novel environment, which is likely to include parameters that are different from their *in situ* "natural ecological" niche, such as light conditions, humidity, diversity and intensity of fungal/mycorrhizal association, diversity and presence and intensity of pathogens. In *ex situ* collections, reproduction/fruit set is likely to be manipulated, and thus reproduction is not determined by floral quantitative characteristics through pollinator selection. In addition, seed germination is likely to be asymbiotic on a "preferred growth medium". Thus, while in an *ex situ* environment it is likely that artificial selection for varying morphological, eco-physiological and growth conditions is a subset of the total *in situ* niche diversity which could result in change or frequency in the genetics and morphology diversity of the characteristics through time. However, for ecologist and conservation biologist the challenge will always be understanding the diversity of habitat, which include the abiotic and biotic parameters in which a species may prosper, clearly a complex task. Even with the best attempts of *ex situ* conservation, it is likely that the condition will never replicate the natural environment 100%. Consequently, *ex situ* collection is likely to be made up of a subset of the "most robust" plants for an "*ex situ* environmental niche". These "robust plants" in *ex situ* environment maybe maladapted for an *in situ* conservation program.

Since Darwin (1877), the study of the close relationship between the fit of specific pollinators and floral characteristics for successful fruit set or pollinaria removal has been of interest to evolutionary biologists. Most orchids have few pollinators (Ackerman *et al.* 2022, Tremblay 1992) and if they have multiple pollinators often these are from limited functional groups (J. D. Ackerman, pers. comm.). The size of flowers can influence reproductive success in plants in general and in orchids (Charpulat *et al.* 2020, de Jager & Peakall 2019, Juillet & Scopece 2010, Palacio *et al.* 2019, Scopece *et al.* 2017, Trunschke 2018). For example, Tremblay (2006) showed that in the diminutive orchid *Lepanthes rupestris* Stimson the size of column and floral characteristics influences pollinaria removal (positive selection coefficients) and fruit set (negative selection coefficient). Cintrón-Berdecía & Tremblay (2006) evaluated variation in selection across seven *L. rupestris* populations in the same period and showed that directional, stabilizing, and disruptive selection varied among characters and populations. Moreover, selection gradients can also vary across reproductive periods as shown in other orchids (example: Scopece *et al.* 2017). There are examples in orchids that show that morphological quantitative characters may influence reproductive success and ultimately, evolutionary processes (phenotypic selection; Tremblay &

Ackerman 2001) and consequently size of morphological characters does matter. Thus, plants that are to be re-introduced to their *in situ* environment as a conservation approach, their functional morphological traits (assuming pollinator mediated pollination) must match the pollinator characteristics if conservation is to be successful.

Here we explore the morphological variation across time of species in the Orchidaceae that have been cultivated by enthusiasts and evaluate if they show changes in the size of the floral characteristics. If no artificial selection is present, then morphological characteristics should not show any increasing or decreasing trend in time. In addition, for a subset of species in our selected dataset we compared the morphological characteristics of plants in cultivation with those described from natural populations using the species descriptions. We ask two fundamental questions: do orchids show evidence of artificial selection while in *ex situ* cultivation and is the morphological variation (range) of the floral characters of *ex situ* plants within the range of the morphology observed in the natural environment?

Materials and methods.

Database.— The morphological floral characters were garnered from the American Orchid Society (AOS) database OrchidPro “https://op.aos.org” which is a repository of all species and hybrids that have been recognized for their “special” qualities by the AOS. We excluded all award types where the focus is not on plant “quality”. The awards included were: HCC (Highly Commended Certificate), AM (Award of Merit), FCC (First Class Certificate), JC (Judge’s Commendation), CBR (Certificate of Botanical Recognition), CCM (Certificate of Cultural Merit) and CCE (Certificate of Cultural Excellence). No hybrids were included in the analyses. For each species, the morphological size of the following characters were included: dorsal sepal length (DSL), dorsal sepal width (DSW), petal length (PL), petal width (PW), lateral sepal length (LSL), lip length (LL) and number of flowers (NumF). These characters were graphically visualized, and statistical tests to determine if there were significant changes across times were performed. Species with subspecies or varieties are considered as part of the normal variation of the species. The minimal sample size was 11 and ranged up to 247 plants (Table 1). Prior to all analysis, visualization of the data was performed to detect possible outliers or likely errors in the dataset and these data points were excluded. For example, a data point in the database for the sepal width of *Brassavola nodosa* (L.) Lindl. was over 7 cm wide; clearly an error in data entry, as the natural range of width of the sepals is from 2–5mm (Ackerman 2014). Another common unrealistic information that appeared in the database is that characters had zero (0) for the size of a character, when these should be either left blank, or an NA should be added. Sample size varied among species and within species because of missing or excluded data for specific characters. To evaluate the above hypotheses, we selected species of the following genera (*Aerangis* Rchb.f., *Angraecum* Bory, *Brassavola* Lindl., *Brassia* R.Br., *Cattleya* Lindl., *Dendrobium* Sw., *Laelia* Lindl., *Paphiopedilum* Pfitzer, *Phalaenopsis* Blume: Table 1). The species selected were those which had the highest number of awards in those genera, however we did add a few species with small sample size to evaluate if patterns were detectable. Although many species are present in the database only a small subset of species are found frequently across many years.

Statistical analysis.— Quantitative characters that were expected to be normally distributed (such as DSL, DSW, PW, PL, LSL, LL, NumF) were evaluated using a linear model (Field *et al.* 2012), while characters that were counts (NumF) were evaluated using a generalized linear model with a negative binomial or log-normal distribution (Kabacoff 2015). Artificial selection for characters was determined by observing if there was either a positive or negative selection for characters across time (Endler 1986). The negative binomial regression is a generalization of the Poisson regression which is less restrictive and does not assume that the mean and variance are equal (Hilbe 2011), while the log-normal regression is a regression with a log-normal distribution where the “y” values are non-negative (Crow & Shimizu 1987). The choice of using the negative binomial or the log normal was made using Akaike Information Criterion (AIC), the model with the smallest AIC was chosen (Cavanaugh & Neath 2019). Assumptions of the models were evaluated for all analysis.

To determine if specific morphological characters across species were more likely to be influenced by ar-

Table 1. List of species evaluated. The first and last year of the data included in the analyses from the AOS database and the reference used to evaluate the native/natural morphological characteristics. Reference for *in situ* refers to the species description for comparison with *ex situ* plants (Table 8). NA= No data from wild type.

Genera	Specific epithet	First year	Last year	Sample size	Reference for *in situ*
Aerangis	*articulata* (Rchb.f.) Schltr.	1971	2014	13	NA
Aerangis	*biloba* (Lindl.) Schltr.	1969	2017	15	NA
Aerangis	*citrata* (Thouars) Schltr.	1965	2018	15	NA
Aerangis	*hariotiana* (Kraenzl.) P.J.Cribb & Carlsward	1975	2014	11	NA
Aerangis	*luteoalba* (Kraenzl.) Schltr.	1965	2020	37	NA
Angraecum	*distichum* Lindl.	1965	2018	18	NA
Angraecum	*eburneum* Bory	1965	2018	28	NA
Angraecum	*leonis* (Rchb.f.) André	1965	2018	20	Notahianjanahary 2016
Angraecum	*magdalenae* Schltr. & H.Perrier	1972	2016	12	NA
Angraecum	*sesquipedale* Thouars	1972	2021	44	NA
Angraecum	*viguieri* Schltr.	1976	2019	20	NA
Brassavola	*nodosa* (L.) Lindl.	1965	2020	62	Ackerman 2014
Brassia	*gireoudiana* Rchb.f. & Warsz.	1977	2018	27	NA
Brassia	*verrucosa* Lindl.	1977	2015	24	NA
Cattleya	*aclandiae* Lindl.	1965	2019	64	van den Berg 2020
Cattleya	*amethystoglossa* Linden & Rchb.f. ex Warner	1965	2020	79	van den Berg 2020
Cattleya	*aurantiaca* (Bateman) P.N.Don	1965	2020	50	Salazar 1990
Cattleya	*bicolor* Lindl.	1965	2019	30	van den Berg 2020
Cattleya	*brevipedunculata* (Cogn.) Van den Berg	1981	2020	21	NA
Cattleya	*cernua* Beer	1965	2019	55	Buzatto 2010
Cattleya	*coccinea* Lindl.	1965	2020	126	Buzatto 2010
Cattleya	*dormaniana* Rchb.f.	1977	2020	25	NA
Cattleya	*dowiana* Bateman	1988	2019	39	Pupulin 2015
Cattleya	*forbesii* Lindl.	1965	2018	23	NA
Cattleya	*granulosa* Lindl.	1965	2019	37	van den Berg 2020
Cattleya	*harpophylla* (Rchb.f.) Van den Berg	1965	2018	19	NA
Cattleya	*harrisoniana* Bateman ex Lind.	1965	2018	28	van den Berg 2020
Cattleya	*intermedia* Graham	1977	2019	72	NA
Cattleya	*jongheana* (Rchb.f.) Van den Berg	1978	2019	48	NA
Cattleya	*labiata* Lindl.	1952	2018	29	NA
Dendrobium	*cuthbertsonii* F.Muell.	1984	2020	117	NA
Dendrobium	*farmeri* Paxton	1965	2021	46	NA
Dendrobium	*lindleyi* Steud.	1965	2021	73	NA
Laelia	*anceps* Lindl.	1974	2020	144	Jiménez *et al.* 1997
Paphiopedilum	*armeniacum* S.C.Chen & F.Y.Liu	1983	2019	152	NA

Table 1. *continues…*

Paphiopedilum	*bellatulum* (Rchb.f.) Stein.	1965	2019	220	Cribb 1987
Paphiopedilum	*charlesworthii* (Rolfe) Pfizer	1977	2019	62	Cribb 1987
Paphiopedilum	*concolor* (Bateman) Pfitzer	1965	2016	60	Cribb 1987
Paphiopedilum	*delenatii* Guillaumin	1965	2019	129	NA
Paphiopedilum	*fairieanum* (Lindl.) Stein	1973	2019	110	NA
Paphiopedilum	*godefroyae* Stein	1973	2019	89	NA
Paphiopedilum	*hirsutissimum* (Lindl. ex Hook) Stein	1975	2002	56	NA
Paphiopedilum	*lowii* (Lindl.) Stein	1975	2014	141	Cribb 1987
Paphiopedilum	*macranthum* Z.J.Lui & S.C.Chen	1985	2018	247	Cribb 1987
Paphiopedilum	*malipoense* S.C.Chen & Z.H.Tsi	1986	2019	108	Cribb 1987
Paphiopedilum	*niveum* (Rchb.f.) Stein	1970	2019	99	Cribb 1987
Paphiopedilum	*philippinense* (Rchb.f.) Stein	1973	2019	81	Cribb 1987
Paphiopedilum	*rothschildianum* (Rchb.f.) Stein	1965	2019	233	NA
Paphiopedilum	*sukhakulii* Schoser & Senghas	1965	2019	208	NA
Paphiopedilum	*venustum* (Wall. ex Sims) Pfitzer	1982	2020	46	NA
Paphiopedilum	*wardii* Summerh.	1972	2016	103	NA
Phalaenopsis	*amboinensis* J.J.Sm.	1969	2020	84	NA
Phalaenopsis	*bellina* (Rchb.f.) Christenson	1969	2021	148	NA
Phalaenopsis	*cornu-cervi* (Hasselt ex Hassk) Blume & Rchb.f.	1972	2021	100	Sweet 1980
Phalaenopsis	*equestris* (Schauer) Rchb.f.	1971	2019	104	Sweet 1980
Phalaenopsis	*lueddemanniana* Rchb.f.	1970	2016	51	Sweet 1980
Phalaenopsis	*pulcherrima* (Lindl) J.J.Sm.	1984	2021	128	NA
Phalaenopsis	*violacea* hort. ex H.Witte	1969	2021	131	Sweet 1980

Table 2. *Aerangis*: Linear coefficients (slope (beta) and standard deviation, SD) of the morphological and floral characteristics across time. NA = not evaluated. The characters included are: dorsal sepal length (DSL), dorsal sepal width (DSW), petal length (PL), petal width (PW), lateral sepal length (LSL), lip length (LL), and number of flowers (NumF). The negative binomial regression (^{a}NB) or Log Normal (^{b}LN) regression were used because the number of flowers has a distribution which is often overdispersed (Cameron & Triverdi 1998) with most plants having few flowers and a few having large number of flowers; significant levels * p<0.05, ** p<0.01 and *** p<0.001. Values in **bold blue** are significant negative coefficients; values in **bold black** are significant positive coefficients, values in regular are non-significant coefficients.

Species	**DSL**	**DSW**	**PL**	**PW**	**LSL**	**LL**	**NumF**
A. articulata	0.008 (0.018)	0.002 (0.002)	0.010 (0.014)	0.003 (0.005)	-0.009 (0.007)	0.011 (0.014)	**0.022^{a} (0.009)***
A. biloba	0.028 (0.016)	0.010 (0.005)	0.025 (0.014)	0.009 (0.004)	**0.011 (0.004)***	0.020 (0.020)	**0.026^{a} (0.011)***
A. citrata	**0.012 (0.006)***	0.008 (0.007)	0.010 (0.005)	**0.008 (0.003)***	0.003 (0.006)	0.008 (0.005)	-0.008^{a} (0.014)
A. hariotiana	0.002 (0.003)	< 0.001 (0.002)	0.002 (0.002)	< 0.001 (0.002)	0.001 (0.002)	< 0.001 (0.003)	NA
A. luteoalba	**0.020 (0.005)*****	**0.001 (0.003)****	**0.018 (0.006)****	**0.013 (0.004)*****	**0.009 (0.003)****	**0.020 (0.008)***	0.004^{a} (0.010)

Table 3. *Angreacum*: Linear coefficients (beta and standard deviation, SD) of the morphological and floral characteristics across time. NA= not evaluated. The characters included are dorsal sepal length (DSL), dorsal sepal width (DSW), petal length (PL), petal width (PW), lateral sepal length (LSL), lip length (LL), number of flowers (NumF). See Table 2 legend for statistical details.

Species	**DSL**	**DSW**	**PL**	**PW**	**LSL**	**LL**	**NumF**
A. distichum	-0.003 (0.002)	-0.001 (0.001)	-0.004 (0.002)	-0.001 (0.002)	0.002 (0.002)	-0.003 (0.002)	0.002[a] (0.025)
A. eburneum	-0.005 (0.009)	-0.003 (0.004)	0.123 (0.161)	-0.016 (0.011)	-0.005 (0.004)	-0.006 (0.011)	0.010[a] (0.020)
A. leonis	0.005 (0.015)	0.003 (0.003)	0.003 (0.014)	0.004 (0.004)	-0.013 (0.014)	0.056 (0.038)	< -0.0001[a] (0.013)
A. magdalenae	0.010 (0.028)	-0.011 (0.012)	-0.009 (0.017)	-0.005 (0.008)	-0.004 (0.007)	0.009 (0.019)	**0.065** **(0.008)*****
A. sesquipedale	-0.022 (0.013)	**0.013** **(0.006)***	-0.011 (0.012)	**0.018** **(0.006)****	**0.013** **(0.005)***	0.061 (0.062)	-0.017[a] (0.010)
A. viguieri	-0.006 (0.011)	0.003 (0.003)	0.014 (0.008)	-0.002 (0.002)	0.002 (0.002)	0.022 (0.033)	**0.035** **(0.010)*****

tificial selection, we summarized the frequency of species which had significant coefficients (either positive, negative or no selection) and evaluated if the frequencies are similar among morphological characters using a Fisher's Exact Test with Monte Carlo simulations (Kassambara 2021).

A comparison of the dispersion (range) in morphological characters between *ex situ* and *in situ* plants was done visually noting the range of morphological characters from the description of the species (see Table 1 for the references for species *in situ* characters) compared to the observed size of plants in the database. If all morphological characters were within the *in situ* range, we coded as "W" = within, if plants grown in *ex situ* collections were above the normal range we coded as "+O" (+ outside) and below the normal range as "-O" (- outside), and if both outside ranges were noted these were scored as "±O". Only a subset of the species in Table 1 were evaluated. This is because the available "species description" were often scarce for many species and the information was limited or did not include all characters evaluated in the previous section. In addition, if sample size were small for an *ex situ* species these characters were excluded.

All analyses were performed with R (v. 4.1.2) using RStudio (v. 2021.09.0, RStudio Team 2020) and the following packages: ggplot2 (Wickham 2016), lubridate (Grolemund & Wickham 2011), janitor (Firke 2021), stringr (Wickham 2019), and dplyr (Wickham *et al.* 2022), gmodel (Warnes *et al.* 2018), rstatix (Kassambara 2021) for data wrangling and statistics, and huxtables (Hugh-Jones 2021) to create the tables.

Results.

Do we observe changes in floral morphology in time?— We evaluated seven morphological characters from 54 species from 9 genera (Table 2–7). Some characters showed significant differences in size across time suggesting that species may be selected artificially for being either larger, such as the DSW in *Phalaenopsis pulcherrima* (Lindl.) J.J.Sm. (Table 7, Fig. 1B) and the lip length in *Angraecum sesquipedale* Thouars (Table 3, Fig. 1E). While other species showed patterns of being smaller as in the PL in *Phalaenopsis violacea* hort. ex Witte (Table 7, Fig. 1A), and *Brassia verrucosa* Lindl. (Table 4, Fig. 1C), and the NumF in *Cattleya granulosa* Lindl. (Table 5, Fig. 1F). While many characters showed no significant change such as the LL in *Dendrobium cuthbertsonii* F.Muell. (Table 4, Fig. 1D).

In total, we evaluated 381 characters, and the proportion of characters that showed significant trends either positive or negative varied among genera (Table 8). As a general trend, characters were more likely to show a positive coefficient (27% across all species) as compared to a negative coefficient (8% across all species) suggesting that morphological change, if significant, were more likely to be larger than when first surveyed. Of the 54 species surveyed only ten species showed no significant change in any of the characters.

TABLE 4. *Brassavola (Bv.), Brassia, Dendrobium and Laelia*: Linear coefficients (beta and standard deviation, SD) of the morphological and floral characteristics across time. NA= not evaluated. The characters included are dorsal sepal length (DSL), dorsal sepal width (DSW), petal length (PL), petal width (PW), lateral sepal length (LSL), lip length (LL), number of flowers (NumF). See Table 2 legend for statistical details.

Species	**DSL**	**DSW**	**PL**	**PW**	**LSL**	**LL**	**NumF**
Bv. nodosa	0.03 (0.16)	**0.004** **(0.002)***	0.011 (0.013)	**0.002** **(0.001)***	0.012 (0.015)	0.002 (0.013)	-0.08[a] (0.18)
B. gireoudiana	-0.066 (0.036)	-0.001 (0.002)	0.022 (0.032)	0.001 (0.001)	-0.100 (0.053)	-0.006 (0.013)	**0.32[a]** **(0.15)***
B. verrucosa	**-0.20** **(0.06)***	-0.005 (0.003)	**-0.29** **(0.097)***	-0.006 (0.003)	**-0.17** **(0.07)***	**-0.076** **(0.021)****	**0.11** **(0.20)*****
D. cuthbertsonii	0.004 (0.004)	0.003 (0.003)	-0.005 (0.004)	< 0.001 (0.003)	0.006 (0.004)	-0.035 (0.021)	-0.016[a] (0.013)
D. farmeri	-0.009 (0.006)	-0.002 (0.004)	-0.010 (0.005)	**0.008** **(0.003)***	-0.002 (0.004)	-0.006 (0.004)	0.005[a] (0.008)
D. lindleyi	< -0.001 (0.004)	-0.003 (0.003)	< -0.001 (0.003)	0.002 (0.003)	0.003 (0.003)	< 0.001 (0.004)	0.010[a] (0.013)
L. anceps	-0.004 (0.007)	**0.008** **(0.003)***	-0.005 (0.006)	**0.015** **(0.006)***	NA	0.005 (0.006)	**0.029** **(0.009)****

Thus, 81% of the species had at least one character that showed a significantly negative or positive trend. Two species had all the evaluated characters with significant trends: *Paphiopedilum malipoense* S.C.Chen & Z.H.Tsi and *Phalaenopsis pulcherrima* (Lindl.) J.J.Sm. (Table 6–7). The only species where selection for smaller size was noted for two or more characters were in *Brassia verrucosa* (Table 4), *Cattleya labiata* Lindl. (Table 5), *Phalaenopsis lueddemanniana* Rchb.f. and *Phalaenopsis violacea* (Table 7).

Artificial selection by type of morphological character.— Across all species some characters were more likely to be selected (Table 8). The DSW, PW and the NumF had the highest frequency of positive coefficients (45%, 36% and 35% respectively). There is little evidence of positive or negative selection on LL (10% and 2% respectively) as 88% of species evaluated did not show any evidence of selection on this character. Most species did not show consistent evidence of artificial selection on some of the morphological characters such as DSL, PL and LSL (69%, 72% and 80% respectively). Approximately 35% of the species showed a positive trend in the NumF and 26% a negative trend (Table 8). The proportion of variables that are significantly different (positive or negative) to those which did not, is not a random distribution across variables. The frequency of LL and NumF often showed significant differences (Fisher's Exact test with simulation, $p < 0.01$). Most pairwise comparisons of the variables show that the proportion of species were not significant. However, the frequency of species that were significant as for LL was significantly different from DSL, PL and NumF (pairwise Fisher comparison test, all p's < 0.0001). The main character which did not follow the same trend in the number of species that show selection coefficient is the LL (rowwise paired Fisher comparison test, $p < 0.0001$).

Is artificial selection consistent among genera?— Variation in size across time was different among genera (Table 9). Considering the species with the greatest number of characters evaluated (*Aerangis*, *Angraecum*, *Cattleya*, *Dendrobium*, *Paphiopedilum*, and *Phalaenopsis*) the frequency of the number of variables was not equal among genera ($p < 0.0001$, Fisher's Exact Test). The genera which diverged the most were *Angraecum* ($p = 0.01$), *Cattleya* ($p = 0.04$) and *Paphiopedilum* ($p < 0.0001$; Fisher's rowwise test). Of the genera where more than one species was evaluated, *Aerangis* (32%) and *Paphiopedilum* (54%) had more characters that showed change in time, while *Angraecum* (12%) and *Dendrobium* (14%) showed fewer characters under artificial selection (Table 9). Considering both negative and positive selection coefficients, the genera which have the least amount of evidence for artificial selection are *Angreacum* (88%) and *Dendrobium* (86%). The genus with the most fre-

Table 5. *Cattleya*: Linear coefficients (beta and standard deviation, SD) of the morphological and floral characteristics across time. NA= not evaluated. The characters included are dorsal sepal length (DSL), dorsal sepal width (DSW), petal length (PL), petal width (PW), lateral sepal length (LSL), lip length (LL), number of flowers (NumF). See Table 2 legend for statistical details.

Species	DSL	DSW	PL	PW	LSL	LL	NumF
C. aclandiae	-0.003 (0.006)	0.01 (0.003)**	-0.006 (0.005)	0.006 (0.003)	-0.008 (0.006)	0.0005 (0.006)	0.012 (0.007)* NB
C. amethystoglossa	0.009 (0.007)	0.007 (0.004)	0.01 (0.007)*	0.02 (0.006)**	0.009 (0.005)	0.005 (0.005)	0.0005 (0.005)*** NB
C. aurantiaca	-0.01 (0.007)	0.009 (0.002)***	-0.01 (0.007)	0.005 (0.003)	0.003 (0.007)	0.0002 (0.006)	-0.017 (0.01)*** NB
C. bicolor	0.005 (0.01)	-0.0006 (0.003)	0.004 (0.009)	0.002 (0.008)	0.002 (0.01)	0.004 (0.006)	-0.007 (0.007)*** NB
C. brevipedunculata	0.03 (0.01)**	0.01 (0.007)	0.04 (0.009)***	0.02 (0.01)	0.02 (0.01)	0.02 (0.007)*	0.03 (0.02)* NB
C. cernua	0.008 (0.003)*	0.005 (0.002)**	0.004 (0.003)	0.002 (0.003)	0.007 (0.003)*	-0.0002 (0.002)	-0.0002 (0.007)*** NB
C. coccinea	0.003 (0.003)	0.008 (0.002)***	0.001 (0.003)	0.02 (0.004)***	0.002 (0.003)	-0.001 (0.002)	0.008 (0.008)** NB
C. dormaniana	0.02 (0.008)	0.001 (0.002)	0.01 (0.007)	0.002 (0.003)	0.009 (0.01)	0.008 (0.006)	0.07 (0.02) NB
C. dowiana	0.05 (0.02)**	0.01 (0.006)	0.01 (0.02)	0.02 (0.01)	0.008 (0.01)	0.009 (0.02)	-0.015 (0.012)*** NB
C. forbesii	0.002 (0.01)	0.004 (0.006)	-0.002 (0.01)	0.006 (0.007)	0.004 (0.01)	0.007 (0.02)	0.04 (0.02)* NB
C. granulosa	-0.01 (0.01)	0.003 (0.003)	-0.004 (0.008)	-0.0002 (0.004)	-0.004 (0.009)	0.009 (0.009)	-0.02 (0.006)*** NB
C. harpophylla	0.002 (0.01)	-0.002 (0.003)	0.005 (0.01)	0.001 (0.002)	-0.01 (0.01)	0.02 (0.009)*	0.02 (0.01)* NB
C. harrisoniana	0.005 (0.009)	0.007 (0.004)	0.001 (0.01)	0.02 (0.01)	0.007 (0.01)	0.01 (0.008)	0.004 (0.009) **NB
C. intermedia	-0.004 (0.01)	0.01 (0.004)*	-0.003 (0.01)	0.02 (0.009)**	0.02 (0.01)	-0.004 (0.008)	-0.0004 (0.02)*** NB
C. jongheana	0.01 (0.01)	0.006 (0.002)**	0.01 (0.008)	0.02 (0.007)*	0.01 (0.009)	0.01 (0.007)	0.01 (0.01)* NB
C. labiata	-0.04 (0.02)*	-0.02 (0.008)*	-0.04 (0.01)**	-0.04 (0.02)**	-0.05 (0.03)*	-0.03 (0.02)	0.007 (0.02)** NB

quent evidence of negative selection is *Brassia*. However, only 2 species were evaluated, and all negative coefficients were detected in a single species, *Brassia verrucosa* (Table 4).

Comparing morphological variation of species description (in situ) and ex situ species.— Plant size range from the *ex situ* grown species are frequently different from plant size of species morphological character noted in the literature. We evaluated 125 characters in 23 species of four genera (*Aerangis*, *Brassavola*, *Paphiopedilum*, *Phalaenopsis*). Plants in cultivation mostly had characters that were larger than the species description (Table 10, Fig. 2). Some character observed from the *ex situ* collections were within the range of the species description (PL in *Brassavola nodosa* Fig. 2A), while many species had characters above the *in situ* range, such as NumF in *Cattleya granulosa* (Fig. 2B), DSW in *C. granulosa* (Fig. 2C), DSL in *C. granulosa* (Fig. 2D) and *C. aclandiae* Lindl. (Fig. 2E), and PW as in *Paphiopedilum macranthum* Z.J.Liu & S.C.Chen (Fig. 2H). While other characters were below the normal *in situ* range such as the LSL in *Phalaenopsis violacea* (Fig. 2F). Some morphological

Table 6. *Paphiopedilum*: Linear coefficients (beta and standard deviation, SD) of the morphological and floral characteristics across time. NA= not evaluated. The characters included are dorsal sepal length (DSL), dorsal sepal width (DSW), petal length (PL), petal width (PW), lateral sepal length (LSL), lip length (LL), number of flowers (NumF). See Table 2 legend for statistical details.

Species	DSL	DSW	PL	PW	LSL	LL	NumF
P. armeniacum	**0.027 (0.006)*****	**0.019 (0.005)*****	0.023 (0.005)	**0.023 (0.006)*****	NA	0.005 (0.013)	0.004[a] (0.009)
P. bellatulum	**0.019 (0.003)*****	**0.019 (0.002)*****	**0.019 (0.003)*****	**0.017 (0.002)*****	NA	0.026 (0.016)	**-0.007[b] (0.003)***
P. charlesworthii	**-0.016 (0.007)***	-0.003 (0.010)	0.005 (0.006)	**0.012 (0.005)***	NA	NA	-0.004[a] (0.009)
P. concolor	-0.004 (0.005)	**0.016 (0.005)*****	0.003 (0.005)	0.006 (0.004)	NA	NA	**-0.318[a] (0.010)****
P. delenatii	**0.016 (0.003)*****	**0.020 (0.003)*****	**0.019 (0.003)*****	**0.027 (0.004)*****	NA	NA	< -0.001[a] (0.007)
P. fairieanum	0.007 (0.004)	**0.019 (0.005)*****	<-0.001 (0.006)	0.006 (0.004)	NA	0.011 (0.015)	**-0.045[a] (0.007)*****
P. godefroyae	**0.016 (0.004)*****	**0.025 (0.004)*****	0.004 (0.004)	**0.013 (0.005)****	NA	NA	0.001[b] (0.003)
P. hirsutissimum	0.003 (0.012)	-0.009 (0.009)	0.015 (0.015)	-0.003 (0.011)	NA	NA	0.015[a] (0.019)
P. lowii	0.046 (0.041)	**0.022 (0.005)*****	**0.028 (0.013)***	0.017 (0.010)	NA	0.038 (0.005)	**-0.012[a] (0.007)****
P. macranthum	**0.013 (0.003)*****	0.005 (0.015)	**0.012 (0.003)*****	**0.015 (0.004)*****	NA	0.023 (0.021)	**0.017[a] (0.007)***
P. malipoense	**0.020 (0.10)***	**0.018 (0.007)***	**0.023 (0.010)****	**0.025 (0.008)****	NA	**0.077 (0.028)***	**0.027[a] (0.010)****
P. niveum	**0.012 (0.003)****	**0.021 (0.004)*****	**0.015 (0.004)****	**0.015 (0.003)*****	NA	0.018 (0.015)	-0.005[a] (0.007)
P. philippinense	0.021 (0.012)	0.006 (0.004)	0.025 (0.035)	0.012 (0.012)	NA	-0.028 (0.017)	-0.010[a] (0.005)
P. rothschildianum	0.011 (0.006)	**0.037 (0.004)*****	**0.049 (0.015)****	0.003 (0.012)	NA	0.003 (0.011)	0.002[a] (0.003)
P. sukhakulii	**0.015 (0.003)*****	**0.009 (0.002)*****	-0.004 (0.008)	0.008 (0.005)	NA	0.005 (0.012)	**-0.012[a] (0.005)* NB**
P. venustum	-0.003 (0.007)	**0.013 (0.006)***	-0.003 (0.007)	0.008 (0.005)	NA	NA	**-0.003[a] (0.017)***
P. wardii	0.001 (0.006)	**0.019 (0.004)*****	0.016 (0.009)	0.006 (0.008)	NA	0.006 (0.028)	0.002 (0.010)

characters when grown *ex situ* the variation was below and above the *in situ* range as in *Paphiopedilum malipoense* (Fig. 2G).

Only in five cases did we identify species with morphological character in the database that were within (W) the described morphology of wild individuals (i.e, PL in *Brassavola nodosa*, LL in *Paphiopedilum bellatulum* (Rchb.f.) Stein, see Table 10), which encompasses only 4% of the variables evaluated. *Ex situ* grown species were observed to have either larger morphological characters than those found in natural populations (75%), smaller (2%) or both (19%). Overall, 96% (120 of 125) of the evaluated characters of *ex situ* plant morphology were sometimes outside the range of plant description from the literature (Table 10).

Considering only the three genera with the greatest number of species, *Paphiopedilum* (8 spp) *Cattleya* (9 spp) and *Phalaenopsis* (4 spp), we observed significant differences in the number of characters which were in the different categories. The main difference was in those in the normal range of the species with 79% in

TABLE 7. *Phalaenopsis*: Linear coefficients (beta and standard deviation, SD) of the morphological and floral characteristics across time. NA= Not evaluated. The characters included are dorsal sepal length (DSL), dorsal sepal width (DSW), petal length (PL), petal width (PW), lateral sepal length (LSL), lip length (LL), number of flowers (NumF). See Table 2 legend for statistical details.

Species	**DSL**	**DSW**	**PL**	**PW**	**LSL**	**LL**	**NumF**
P. amboinensis	0.004 (0.003)	**0.007** **(0.002)****	**0.004** **(0.002)***	0.002 (0.002)	0.004 (0.003)	0.002 (0.003)	**-0.009**[a] **(0.009)***
P. bellina	-0.003 (0.003)	**0.009** **(0.001)*****	-0.0005 (0.002)	**0.008** **(0.002)*****	0.002 (0.003)	0.004 (0.002)	**-0.022**[a] **(0.004)*****
P. cornu-cervi	-0.004 (0.004)	0.003 (0.002)	-0.003 (0.003)	-0.002 (0.002)	-0.002 (0.004)	< -0.001 (0.003)	-0.020[a] (0.010)
P. equestris	0.0008 (0.003)	-0.0004 (0.002)	0.0005 (0.005)	-0.002 (0.003)	-0.0005 (0.003)	-0.004 (0.003)	**0.018**[a] **(0.009)***
P. lueddemanniana	-0.005 (0.004)	0.003 (0.002)	**-0,008** **(0.003)***	**0.007** **(0.003)***	**-0.008** **(0.004)***	-0.002 (0.002)	-0.008[a] (0.010)
P. pulcherrima	**0.008** **(0.003)***	**0.011** **(0.002)*****	**0.011** **(0.003)*****	**0.027** **(0.005)*****	**0.011** **(0.003)****	**0.011** **(0.004)****	**0.022**[a] **(0.007)****
P. violacea	**-0.009** **(0.002)*****	0.0008 (0.001)	**-0.012** **(0.002)*****	0.0004 (0.0017)	0.011 (0.06)	-0.004 (0.003)	**-0.017**[a] **(0.006)****

TABLE 8. Summary of the proportion of species where characters were significantly different (p< 0.05) across time (column wise proportions). The selection coefficients are in Table 2a-f. Values in brackets are the number of species. The characters included are dorsal sepal length (DSL), dorsal sepal width (DSW), petal length (PL), petal width (PW), lateral sepal length (LSL), lip length (LL), number of flowers (NumF). Note sample size (number of species) varies among characters.

Species	**DSL**	**DSW**	**PL**	**PW**	**LSL**	**LL**	**NumF**
Positive effect	0.24 (14)	0.45 (26)	0.21 (12)	0.36 (21)	0.12 (5)	0.10 (5)	0.35 (20)
Negative effect	0.07 (4)	0.02 (1)	0.07 (4)	0.02 (1)	0.08 (3)	0.02 (1)	0.26 (15)
No significant selection	0.69 (40)	0.53 (31)	0.72 (42)	0.62 (36)	0.80 (32)	0.88 (46)	0.39 (22)

TABLE 9. Summary of proportion of characters by genera which show significant change in time (rowwise). Positive= Proportion of characters with significant positive change in time. Negative= Proportion of characters with significant negative change in time. No selection= Proportion of characters with no significant change (see Table 2–7).

Genus (number of species)	**Number of characters evaluated**	**Positive**	**Negative**	**No selection**
Aerangis (5)	33	0.30	0	0.70
Angraecum (6)	42	0.12	0	0.88
Brassavola (1)	7	0.29	0	0.71
Brassia (2)	14	0.14	0.29	0.57
Cattleya (16)	112	0.24	0.10	0.66
Dendrobium (3)	21	0.05	0	0.95
Laelia (1)	6	0.5	0	0.5
Paphiopedilum (17)	96	0.41	0.07	0.52
Phalaenopsis (7)	49	0.27	0.14	0.59

Paphiopedilum, 79% in *Cattleya* and 57% in *Phalaenopsis* (Fisher's Exact test with Monte Carlo simulation $p < 0.02$; Table 11). *Cattleya* had 22% of the characters which were smaller and larger than species description. This suggests that the selection for characters differences from *in situ* may differ among genera.

The range of morphological characters in *ex situ* plants were often outside and larger than the range of *in situ* plant description (range 64–95%) for all characters except LL (36%). LL for some species had either

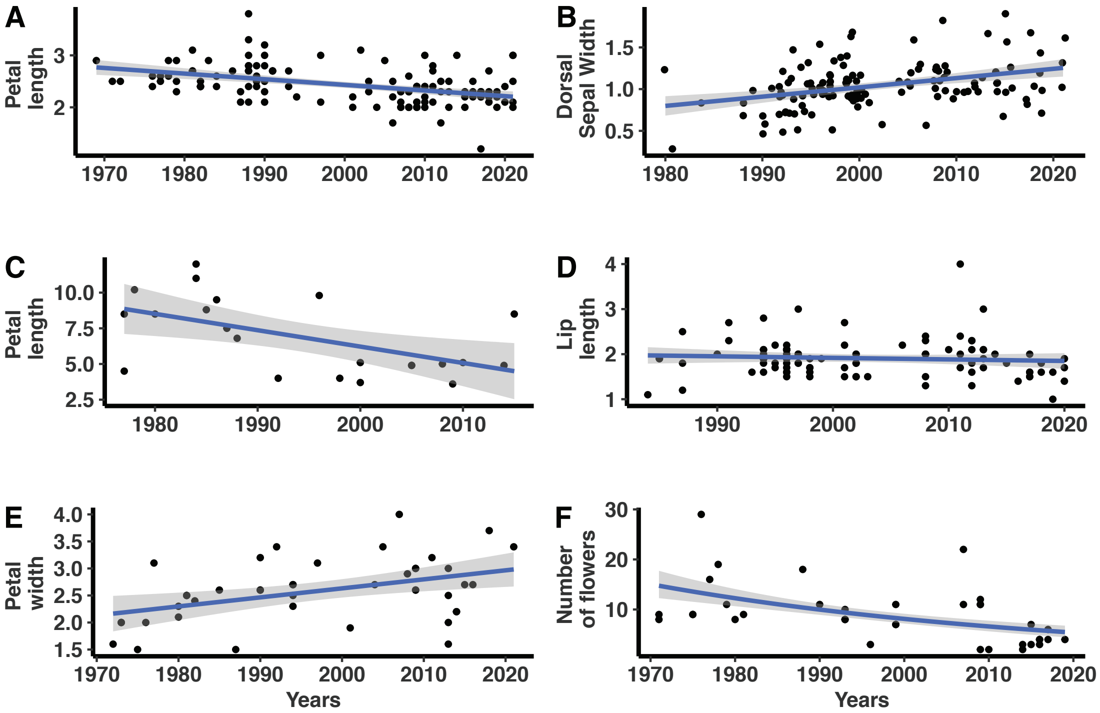

FIGURE 1. Examples of typical trends in morphological variation in time. Each analysis in Table 2 was evaluated visually to identify outliers (which were excluded) prior to statistical analysis. The blue line represents the "best" slope of the model and the shaded area represents the 95% confidence interval of the slope. **A**. Variation in petal length in *Phalaenopsis violacea*, significant negative linear slope. **B**. Dorsal sepal width variation in *Phalaenopsis pulcherrima*, significant positive linear slope. **C**. Petal length variation in *Brassia verrucosa*, significant negative linear slope. **D**. Lip length in *Dendrobium cuthbertsonii*, slope not significant. **E**. Petal width variation in *Angraecum sesquipedale*, significant positive slope. **F**. Number of flower variation in *Cattleya granulosa*, significant negative binomial slope.

larger and smaller morphological size (43%), showing an inconsistency compared to other characters (Table 12). Note that sample size and characters evaluated is different from previous analysis as gathering quality data of *in situ* morphological characters were often challenging. As an example, *Angraecum leonis* (Rchb.f.) André only data on NumF was considered of confidence (Table 11).

Discussion.

Artificial selection.— In general, we noted that plants that are grown *ex situ* frequently show a trend of either increasing or decreasing size in time. This index suggests that there is artificial selection for morphological characteristics of orchid flowers by orchid enthusiasts. The presence or lack of artificial selection in some characters appears to be more consistent across species. Some characters seem to have little to no evidence of artificial selection, such as LL. The lack of evidence of artificial selection in some characters is likely to be attributed to the lack of interest by growers for that characteristic or alternatively little genetic diversity within species for that specific characteristic may be present. Alternatively, the size of the lip (length and width) is likely to be highly correlated with the fitness of the individual *in situ*, as the relationship between pollinator size and lip size is likely to be important. Therefore, there may be selection against variation (stabilizing selection) and thus selection for reduced genetic diversity and consequently, phenotypic variation. If growers were interested in modifying lip size, the artificial selection process may be more tedious.

Other characteristics such as DSW and PW do show evidence of change across time in many spe-

TABLE 10. Comparison of morphological characteristics of morphology from plants in their natural environment (*in situ*) vs. *ex situ* plants. The morphological characters of plants in the database are considered either W= within the natural morphological range, O= outside the natural morphological range with "+" values larger than reported in nature and "-" values smaller than reported in nature, "--" for species where the character was not evaluated. The morphological range was collected from species description (see Table 1 for references). The characters included are dorsal sepal length (DSL), dorsal sepal width (DSW), petal length (PL), petal width (PW), lateral sepal length (LSL), lip length (LL), number of flowers (NumF).

Species	**DSL**	**DSW**	**PL**	**PW**	**LSL**	**LL**	**NumF**
Angreacum leonis	--	--	--	--	--	--	+O
Brassavola nodosa	+O	+O	W	+O	+O	+O	+O
Cattleya aclandiae	+O	+O	+O	+O	+O	+O	+O
Cattleya amethystoglossa	+O	+O	+O	+O	+O	+O	+O
Cattleya aurantiaca	+O	+O	±O	+O	+O	±O	+O
Cattleya bicolor	±O	+O	±O	+O	+O	±O	+O
Cattleya cernua	+O	+O	+O	+O	--	--	+O
Cattleya coccinea	+O	+O	+O	+O	+O	--	+O
Cattleya dowiana	--	--	±O	+O	--	+O	+O
Cattleya granulosa	±O	+O	±O	+O	±O	±O	+O
Cattleya harrisoniana	--	--	±O	+O	--	±O	+O
Paphiopedilum bellatulum	+O	+O	+O	+O	--	W	+O
Paphiopedilum charlesworthii	±O	+O	±O	+O	--	--	+O
Paphiopedilum concolor	+O	+O	+O	+O	--	--	+O
Paphiopedilum lowii	+O	+O	+O	W	--	--	+O
Paphiopedilum macranthum	W	+O	+O	+O	--	±O	+O
Paphiopedilum malipoensis	+O	+O	+O	±O	--	W	+O
Paphiopedilum niveum	+O	+O	+O	+O	--	--	+O
Paphiopedilum philippinense	+O	+O	+O	--	--	--	±O
Phalaenopsis cornu-cervi	±O	±O	+O	±O	±O	--	--
Phalaenopsis equestris	+O	+O	+O	+O	±O	+O	--
Phalaenopsis lueddemanniana	+O	+O	--	--	+O	±O	--
Phalaenopsis violacea	+O	+O	±O	+O	-O	-O	--

cies or frequently within a specific genus. The DSW showed a significant change in 14 of 17 species of *Paphiopedilum*. This suggests that growers may be selecting for this characteristic.

NumF was the character that showed the highest artificial selection, with 43% of species showing either a negative or positive change in time. NumF on a plant can drastically influence the reproductive success in the field. For example, in *Cyclopogon elatus* (Sw.) Schltr., where Benitez-Vieyra *et al.* (2006) showed that for small display size there is stabilizing selection. Orchid species studied in the field often show that the larger the floral display the higher reproductive success (pollinaria removal or fruit set; Tremblay 2005 and references within), although in at least one case smaller inflorescence had higher reproductive success (*Ionopsis utricularioides* (Sw.) Lindl., Montalvo & Ackerman 1987). What is the cost of having larger inflorescence for the lifetime reproductive success of an orchid? This topic has rarely been studied in orchids (Calvo 1993, Obeso 2002, Tremblay *et al.* 2005), let alone evaluating if in *ex situ* orchids with larger inflorescence re-established in nature would result in lower fitness because of the extra cost of producing these.

Ex situ vs. in situ morphological range.— Floral characters are frequently either larger or smaller than the species description. This would suggest that growers are selecting for characters outside the "normal range"

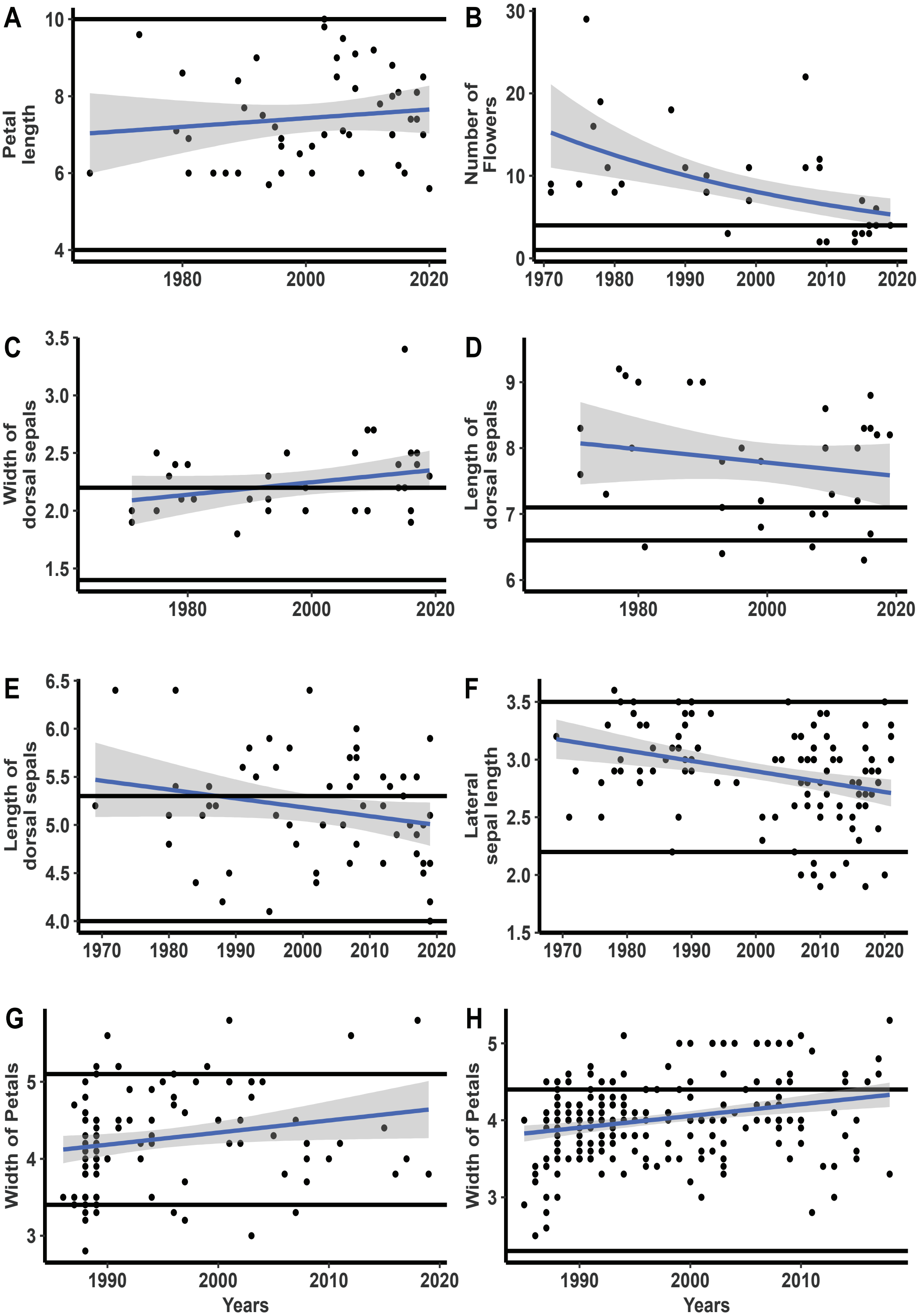
A
Petal length
10
8
6
4
1980
2000
2020
B
Number of Flowers
30
20
10
0
1970
1980
1990
2000
2010
2020
C
Width of dorsal sepals
3.5
3.0
2.5
2.0
1.5
1980
2000
2020
D
Length of dorsal sepals
9
8
7
6
1980
2000
2020
E
Length of dorsal sepals
6.5
6.0
5.5
5.0
4.5
4.0
1970
1980
1990
2000
2010
2020
F
Lateral sepal length
3.5
3.0
2.5
2.0
1.5
1970
1980
1990
2000
2010
2020
G
Width of Petals
5
4
3
1990
2000
2010
2020
Years
H
Width of Petals
5
4
3
1990
2000
2010
Years

TABLE 11. The proportion of species by genera which had morphological characters either larger "+", smaller "-", both "±" or within "W". The proportions are rowwise. See Table 10 for character description.

Genus	+	-	±	W
Angreacum	1.00 (1)	0.0 (0)	0.0 (0)	0.0 (0)
Brassavola	0.86 (6)	0.0 (0)	0.0 (0)	0.14 (1)
Cattleya	0.78 (42)	0.0 (0)	0.22 (12)	0.0 (0)
Paphiopedilum	0.79 (33)	0.0 (0)	0.12 (5)	0.09 (4)
Phalaenopsis	0.57 (12)	0.10 (2)	0.33 (7)	0.0 (0)

TABLE 12. The proportion of species which had morphological characters either larger "+", smaller "-" or both "±". The proportions are rowwise. See Table 10 for character description. Dorsal sepal length (DSL), dorsal sepal width (DSW), petal length (PL), petal width (PW), lateral sepal length (LSL), lip length (LL), number of flowers (NumF).

Categories	+	-	±	W
DSL	0.75 (15)	0.0 (0)	0.20 (4)	0.5 (1)
DSW	0.95 (19)	0.0 (0)	0.05 (1)	0.0 (0)
PL	0.62 (13)	0.0 (0)	0.33 (7)	0.05 (1)
PW	0.85 (17)	0.0 (0)	0.10 (2)	0.05 (1)
LSL	0.64 (7)	0.09 (1)	0.27 (3)	0.0 (0)
LL	0.36 (5)	0.07 (1)	0.43 (6)	0.14 (2)
NumF	0.95 (18)	0.0 (0)	0.05 (1)	0.0 (0)

of the species. The assumption that the difference between the range of floral characteristics of *ex situ* species and the range in the natural environment (*in situ* diversity) is a "good" representation of the morphological variation of the species in the field should be weighted with caution. This assumption is not likely to hold for many species, as the number of observed plants for the original or posterior species collection and description are frequently limited to few individuals, consequently the species taxonomic description is possibly a reduced subset of the variation in their natural environment. In general, taxonomists rarely mention the number of individual plants they used to describe a species; thus, it is difficult to evaluate if the morphological variation observed represent the varying ecological, temporal and geographical distribution of the species. Even more worrisome is when the selection of the plants in the field as "type specimen" are biased towards specific characteristics such as larger plants/flowers or larger inflorescences. In most cases, none of the prior mentioned drawbacks are available to evaluate critically, and consequently, one has to assume that the description is a "good approximation" of the morphological diversity of species *in situ*. This assumption should be tested to determine if there is a systematic bias in species description as compared to *in situ* plant characteristics.

Phenotypic variation.— The origin of morphological variation (Newman & Müller 2000, Rebeiz & Tsiantis 2017, Salazar-Ciudad 2007) and its evolutionary processes is a complex area of study (Budd 2006, Endler 1986, Pigliucci 2005, Shepard & Purugganan 2002). Three main components which influence morphological variation include genetic variation, environmental conditions and the interaction between genetics and the environment interaction (phenotypic plasticity). Where does most of the variation in floral characteristics come from, and what is the environment, genetics and phenotypic plasticity impact on the total phenotypic variation of a species?

There are constraints to morphological variation and phenotypic plasticity (Muren *et al.* 2015, Snell *et al.* 2010), and its evolutionary significance can be important (Bradshaw 1965). Climate change may also in-

LEFT, FIGURE 2. Examples of morphological variation within or outside the range of *in situ* species. Visual representation of a subset of the species evaluated in Table 10. In each figure the horizontal lines represent the maximum and minimum size of the characters as described in the literature (see Table 1 for references). The blue line is the slope and the shade area the 95% CI (See Table 2). **A**. Variation in petal length in *Brassavola nodosa*, size of petal length of *ex situ* plants is within (W) natural range of the species. **B**. Number of flowers in *Cattleya granulosa*, most of the plants had higher number of flowers than the range described for the species (+O). **C**. Dorsal sepal width in *Cattleya granulosa*, most plants had large DSW than in the literature (+O). **D**. Dorsal sepal length in *Cattleya granulosa*, most plants had large DSL than in the literature (+O). **E**. Dorsal sepal length variation in *Cattleya aclandiae*, many of the plants had large characters (+O). **F**. Lateral sepal length in *Phalaenopsis violacea*, note the many plants which had smaller LSL (-O). **G**. Petal width in *Paphiopedilum maliopoense*, note plants that are larger and smaller than the natural range (±O). **H**. Petal width in *Paphiopedilum macranthum*, note plants that are larger and smaller than the natural range (±O).

fluence phenotypic plasticity (Arnold *et al.* 2019) and this area of study has yet to be explored in orchids. Morphological and colour variation in the wild has been studied in many orchids but has yet to elucidate many of the questions mentioned above (Bateman & Rudall 2006, Blinova 2012, Dormont *et al.* 2019, Shi *et al.* 2009, Tremblay 1997). The plasticity of morphological characters and ability to vary across time is most likely genetically complex, consequently, for some characters it is possible that the selector (growers) was not interested in some specific morphological characters, thus little artificial selection is noted, orienting their focus on other morphological characters. Naturally, it assumes that there is an underlying genetic diversity that can be selected for the specific character of interest and that phenotypic plasticity may play a substantial role in the morphological diversity of plants *in situ* and *ex situ* in orchids.

Studies of phenotypic selection in orchids in the wild are limited, with evidence showing that phenotypic selection is variable in time (Benitez-Vieyra *et al.* 2009) and space (Cintrón-Berdecia & Tremblay 2006, Maad 2000). Not surprisingly evidence of varying phenotypic selection among orchid species is common (Moré *et al.* 2012). The only paper noted in Google Scholar of orchids studying phenotypic plasticity attempted to evaluate the CAM response of photosynthetic behavior in two orchid genera, *Angraecum* and *Microcoelia* Lindl., showing very little variation in CAM response across species (Kluge *et al.* 2001).

Artificial selection and adaptation to natural populations.— We do not know if the change in morphological size observed in *ex situ* plants would result in differential fitness if these species with selected characters that vary from their *in situ* counterparts were to be re-introduced in the native habitat. Nor do we know if these *ex situ* plants would retain those size characteristics *in situ*. Growth conditions *in situ* often reduces stress on plants because resource availability is controlled and is likely to be less constrained than under *ex situ* conditions. However, morphological differences of sometimes minute change in floral characters in many species have been shown to impact reproductive success of plants in general and orchids (Gasket 2012, Juillet & Scopece 2010 and references within). Thus, it is most plausible that these plants would be non-adaptive if morphological characters of *ex situ* plants varied from *in situ* plants and are retained in a re-introduction conservation program. There is multiple evidence that *ex situ* plants do acquire characteristics which are different and potentially less adaptive to *in situ* environments. Lofflin and Kephart (2005) showed that in a re-establishment experiment of the *Silene douglasii* Hook. (Caryophyllaceae) in sympatry with *in situ* plants of the same species, *ex situ* individuals were highly maladapted. This may not need to be the case for all species (Hamelin 2012). In one of the few orchid reintroduction programs, Aggarwal and Zettler (2010) showed a very good survival rate of *Dactylorhiza hatagirea* (D.Don) Soó; however, these seeds were collected from the wild and thus unlikely to have been under artificial selection. They grew the seeds on a growth medium, and this procedure did not appear to have had any effect for differential survival in their two-year survey.

Plants in *ex situ* can shift the mean of traits of some characteristics such as germination, flowering time, and decrease in stress tolerance and also dormancy period (Ensslin *et al.* 2015, 2018, Rauschkolb *et al.* 2019) and even resistance to herbivory (Wang *et al.* 2021). Consequently, when considering plant traits, one should consider not only morphological characteristics but underlying molecular and biochemical characteristics that may influence the reintroduction process. For example, in a very large reintroduction program in Korea of the epiphytic *Dendrobium moniliforme* (L.) Sw., the size of plants changed across 3 years and was dependent of the host tree. Whereas a general trend plants tended to be smaller than when originally introduced; however, in a few host trees species the length of the propagule was larger (Kim *et al.* 2016). In a more recent review of orchid reintroduction and their interactions with orchid mycorrhizal fungi (OMF), the concept of artificial selection (or natural selection) as a process that could influence reintroduction success rate was not even mentioned (Zhao *et al.* 2021); however, the authors clearly propose a protocol that includes many variables that need to be considered and are required for rebuilding sustainable *in situ* populations. One area of research that needs to be explored, if *ex situ* plants are to be introduced to their native habitat, is the relationship between OMF and the fitness of the orchid. In *ex situ* orchids OMF are often absent (Irene

Díaz; pers. comm.) and the impact of this on the fitness of a re-introduction effort is unknown.

Limitations.— Large variation in morphology as a consequence of environmental conditions may result in variation in size which may or may not be heritable. Most quantitative morphological characters are not heritable by simple Mendelian inheritance. In addition, it is most likely that quantitative variation is a consequence of not only genetics but an interaction between genetics and the environment (phenotypic plasticity). Phenotypic plasticity, or at least variation in morphological size of individuals across years can vary enormously (Tremblay & Bould 2017, Tremblay *et al.* 2010) and influence reproductive potential (Tremblay *et al.* 2010). In many, cases, evidence for larger or smaller morphological characters in time of *ex situ* plants were noted, suggesting that *ex situ* species may be under artificial selection. In addition, observation that many plant characters are larger in *ex situ* environments than in nature (Table 8) is likely in part due to higher quality resources and cultivation management as compared to the natural environment. It is possible that plants in nature are most frequently smaller than their genetic potential because of resource limitations. Thus, the morphological variation and range in *in situ* environment may be reduced as compared to *ex situ* plants.

Conclusions. Awarded *ex situ* orchids show ample evidence of artificial selection. *Ex situ* conservation has the main objective of conserving the maximum amount of genetic and morphological variation of any species in the wild until these can be re-introduced in their native habitat. Presumed *ex situ* conservation strategies that change the morphological size and variation, and genetic diversity are likely to fail in *in situ* when these are re-introduced in their native habitat. Artificial selection while in non-native habitat may result in a change in the diversity of genetic diversity and morphology, which might be maladaptive when re-introduced in the wild. Award-winning species are most likely to be a small subset of the diversity of genetic and morphological characteristics of native species and thus should not be perceived as an *ex situ* conservation strategy. What are the morphological and genetic characters which are needed for successful re-introduction of orchids in their native habitat? Limited information on the morphological characters of *in situ* populations of orchids and how this influences fitness in their native habitat is present in the literature. The ecological niche of orchids is likely to be highly complex when considering the complexity of the life history of orchids (Swarts & Dixon 2017). Future studies should be undertaken to evaluate if the hypotheses developed above hold any validity. For example, selecting a species that has been under cultivation and shown to morphologically vary from natural populations and reintroduced to its native habitat to determine if the *ex situ* plants can be successfully integrated to a local population even if they morphologically differ from the *in situ* plants after re-establishment. This is possible if most of the variation in size of *ex situ* plants is a consequence of the environment or phenotypic plasticity, not solely genetically based.

AUTHORSHIP. RLT developed the concept and statistical methods, first draft and analyzed the following genera (*Brassia*, *Brassavola*, *Cattleya*, *Dendrobium*, *Laelia*, *Paphiopedilum*). PAAR analyzed *Paphiopedilum*, AAR analyzed *Aerangis* and *Angraecum*, and SDR analyzed *Phalaenopsis* and *Cattleya.* IMT prepared part of the visualization tables and figures.

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