EVOLUTION IN SMALL POPULATIONS: EVIDENCE FROM THE LITERATURE AND EXPERIMENTAL RESULTS

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The great taxonomic diversity of the Orchidaceae is often attributed to adaptive radiation for specific pollinators driven by selection for outcrossing. However, when one looks beyond the product to the process, the evidence for selection is less than overwhelming. Here we review a number of investigations that attempted to determine if natural selection is present in a variety of orchids based on the data from the literature in addition to our own research to understand the relative importance of this process. We illustrate through these examples that attempts to measure and demonstrate evidence for natural selection reveal selection coefficients that are most often small and non-significant. From the literature we show selection studies for morphological variation in *Lepanthes rupestris* Stimson (Cintrón-Berdecia & Tremblay 2006), unpublished work on selection coefficients for color variation in *Lepanthes rupestris* (Tremblay & Ackerman, submitted), color variation in *Bletia patula* Graham (Ackerman & Carromero 2005) and in *Psychilis monensis Sauleda* (Aragon and Ackerman 2004), and phenotypic selection for size in *Caladenia gracilis* R. Br.

Prior to any studies evaluating the potential for evolution, it is important to determine that the characteristics of interest are heritable. Genetic variation is not the only cause of morphological variation. Morphological variation can be a result of the environmental factors and also by the interaction between genetics and environmental (G X E, phenotypic plasticity). In the studies that follow we assume that most morphological variation is a direct result of genetic variation or genetic by environment interaction and not dominated by environment. Previous results suggest that significant variation attributable to environment can occur in some floral characteristics but others appear relatively unaffected by environmental changes (Morales Vargas 2003).

Size: Is it superficial?

In the first example Cintrón-Berdecia and Tremblay (2006) sought to identify floral characters that suggested evidence for either linear, disruptive or stabilizing selection on flowers of more then 200 individuals of *Lepanthes rupestris* Stimson in seven populations along two river systems. None of the analyses suggested that any of the characters were under selection in all populations. They found scattered evidence that some of the characters had positive reproductive output through either pollinaria removal or fruit set when the floral character was larger (length of column; in two populations; mid lobe length, lip width, posterior petal lobe), while other characters suggested negative effect when characters were larger (width of anterior petal lobe, front lip length, flower size). A few characters appear to be subjected to disruptive selection (length of anterior petal lobe, width of anterior petal lobe, front lip width) and stabilizing selection (length posterior petal lobe, distance between sepals, Table 1). In spite of this the most outstanding result of the survey is the complete inconsistency in significant selection coefficients in any of the characters among populations. The character with the most frequent significant selection coefficient was the length of the column, which was found only in two populations, through both pollinaria removal and fruit set.

When all data from the seven populations are summed and analyzed, the length of the column is again the only character where the linear selection coefficient is significantly different using both measure of reproductive success (larger column length have higher selection coefficient on reproductive success, 6.2% (pollinaria removal) and 5.3% (fruit set). The length of the lip and distance between sepals were also found to be positively correlated with size through pollinaria removal.

TABLE 1: Univariate (*s'*, *ß''*) and multivariate (*γ'*) selection acting through female success (fruit set) fitness on 12 characteristics in one populations of *Lepanthes rupestris* and the sum of all seven populations (populations 2 to 7 not shown; see Cintrón - Berdecia and Tremblay 2006). Standardized selection coefficients are represented as directional (*s'*, *ß'*) and non-lineal (*γ'*, negative values = stabilizing selection, positive values = disruptive selection) and given in units of phenotypic standard deviation. Bold numbers are significant at the following levels, *P £ 0.05, **P £ 0.

| Female Fitness | Population 1 | | | | All Populations | | | |
Traits	n	*s'*	*ß'*	*γ'*	n	*s'*	*ß'*	*γ'*
Length of Column	35	**.189***	.200	.054	201	**.062***	.048	.001
Width Sepal Dorsal	29	.163	.058	-.054	189	.022	-.018	-.001
Length Posterior Petal Lobe	29	.113	-.066	-.141	187	.018	.002	-.001
Width Posterior Petal Lobe	29	.086	.032	-.077	188	.029	.032	-.018
Length Anterior Petal Lobe	29	.131	.043	-.123	189	.044	-.040	-.001
Width Anterior Petal Lobe	29	.218	.122	-.003	189	.010	.058	.003
Front Lip Length	29	.077	.061	-.044	186	**.052***	.043	-.003
Front Lip Width	29	**.439***	.182	.036	186	.012	.009	.011
Mid Lobe Length	26	-.033	-.200	.032	176	.023	.023	-.002
Anther Cap Open	28	-.068	-.204	.043	184	.016	-.092	.011
Distance Between Sepals	27	.040	.058	.008	184	.017*	.007	011
Flower Size	33	.090	.170	-.158	208	.010	-.004	.008

However, fruit set suggests that the distance between sepals was under disruptive selection (Table 1).

Color: Can you see it and do you care?

In the second example Tremblay and Ackerman (unpublished) sought to measure phenotypic selection on petal color variation in populations of *Lepanthes rupestris*. Plants have flowers with either unicolor or bicolored petals. The two color morphs are otherwise inseparable morphologically. Reproductive success is skewed towards few individuals and effective population sizes are estimated to be small. We censused seven populations monthly for 20 months or more and noted flower production and petal color pattern. Each flower was checked for pollinarium removal (a measure of male fitness) and fruit production (a measure of female fitness). In all populations, plants with bicolored petals dominated, comprising 63-82% of individuals. Nevertheless the two types were indistinguishable based on reproductive success. Flower color pattern was generally not associated with either male or female reproductive success within or among populations or over time. Although we were unable to tag fitness to petal color patterns, the consistent ratio of color morphs among populations suggests that factors

other then just drift are responsible for the frequencies we observed within a population.

In food-deceptive orchids, variation in floral characteristics associated with pollinator attraction is expected to be high, largely due to either relaxed selection or negative frequency-dependent selection (Ackerman & Galarza-Pérez 1991). Relaxed selection would occur if flower color made no difference, as may be the case for *Lepanthes rupestris*. Negative frequency dependent selection occurs when being different imparts an advantage because pollinators will more quickly learn that similar phenotypes have no rewards. Ackerman and Carromero (2005) surveyed populations of *Bletia patula* in the Dominican Republic in a region where two color morphs were common: pink flowered and white flowered plants. While no morphological differences between the two color morphs were detected, there were differences in reproductive success between the two. The white-flowered plants had an advantage through male function (they were more likely to have pollinaria removed than pink-flowered plants). However, success was not related to color morph frequencies, neither negatively nor positively. The two color morphs also occur in Puerto Rico, but the white-flowered plants are extremely rare precluding any comparison between populations of the two islands.

When color variation is bimodal, such as in *Lepanthes rupestris* and *Bletia patula*, it is relatively easy to associate a color morph with pollinator behavior and reproductive success. However, in most orchids with substantial variation in floral color the variation is continuous. Under such circumstances it is somewhat more difficult to interpret variation in pollinator behavior and plant reproductive success. In an experimental study Aragón and Ackerman (2004) manipulated color variation in *Psychilis monensis*, creating uniformly and variably colored populations. As plants of this species flower all year, treatments were rotated among populations over time. They found that over 50% of the variation in either male or female reproductive success was explained by time and site with no significant effect of treatment except as part of a three-way interaction of time X site X treatment. Population variation in floral color had little or no effect on reproductive success. Major community changes had occurred during the experiment with flowering activity of sympatric species falling dramatically and by the third run of the experiment, only *P. monensis* was in flower. This is when the number of effective visits significantly increased. They concluded that high natural levels of color variation may be more influenced by drift than selection

Showing off: Size matters

Selection may also act on floral display and thus there is an opportunity for pollinator-mediated selection (Williams & Conner 2001, Kobayashi *et al*. 1997, Worley *et al*. 2000, Totland *et al*. 1998). *Caladenia (Stegostyla) gracilis* R. Br. is a widespread terrestrial orchid from Eastern Australia. Tremblay (2006) surveyed populations at two sites in the state of Victoria.
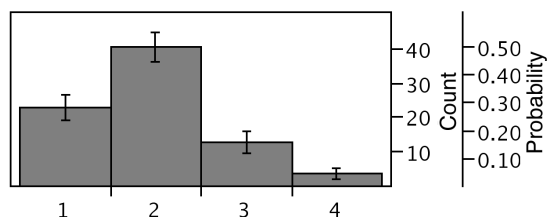

FIGURE 1: Frequency (s.e.) distribution of floral display size in 81 individuals of *Caladenia gracilis* at two sites in the state of Victoria, Australia.

Inflorescences have 1-6 flowers, each about 30 mm across (Tremblay 2005). A total 81 plants at two sites were sampled for reproductive success (pollinaria removed, pollination and fruit set) as a function of number of flowers on a plant. Plants with two flowers was the most common display size at both sites (mean + s.e.; 2.0 ± 0.11; Figure 1).

Plants that have fewer flowers have a very low probability of having their pollinaria removed or deposited or setting fruit. The probability of reproductive potential was significantly higher in multi-flowered individuals (Logistic regression; log likelihood = 8.134, df 1, 80, p < 0.0001: Table 2).

A literature review of some of the evidence of the effect of floral display on reproductive success in orchids suggests that when significant effects occur, reproductive success is higher in larger inflorescences, although it does not necessarily increase proportionately with an increase in the number of flowers (Montalvo & Ackerman 1987). However, in a number of orchid species pollinators appear to have no preference for floral display size (Table 3). The ability to detect a significant effect is sample size dependent, however there is no evidence to suggest that these non-significant results are an aberration. Moreover, the effect of reproductive success on floral display can be

TABLE 2. The expected male and female reproductive success of individuals of *Caladenia gracilis* with varying floral display. Analysis of receiver operating characteristics (ROC) calculates the most likely state of each of the type of floral display from the logistic regression equation. Thus a flower on a two-flowered inflorescence has a 32% chance of having the pollinaria removed, while on a four-flowered inflorescence, an individual flower has an 89% chance of having the pollinaria removed.

Floral display (number of flowers)	Expected percent pollinaria removal	Expected percent pollinaria deposition	Expected percent Fruit set
1	0.11	0.09	0.10
2	0.32	0.17	0.21
3	0.66	0.30	0.37
4	0.89	0.47	0.57

inconsistent among time and space, as it has been shown for other characters (Maad 2000, Ehlers *et al.* 2002; Tremblay & Ackerman, unpublished).

Among the different studies discussed above, no general pattern of selection was observed for color morphs or morphological characters. The selective advantage of floral color could be affected by a number of variables, the first is it assumes that the pollinators can visualize the color variation perceived by humans, secondly it assumes that the color variation is sufficiently discrete so they care enough to make a choice between the variants. Furthermore, the ecological context may make all the difference as well. Some European, rewardless orchids have dramatic flower color polymorphisms as in *Bletia patula*, but unlike the tropical species, color and its frequency have been shown to make a difference to orchid reproductive success (Smithson & Macnair 1997, Gigord *et al.* 2001). Such differences in response to color poly-

TABLE 3: Effect of floral display on reproductive success in orchids. "+" = positive effect of larger floral display on reproductive success, "-" = negative effect of larger floral display on reproductive success, "D" = disruptive selection, NS = no significant effect of floral display on reproductive success.

Species	Variation in number of flowers	Pollinaria removal	Fruit set	References
Aspasia principissa Rchb. f.	1-7	+	+	Zimmerman & Aide 1989
Brassavola nodosa (L.) Lindl.	1-5	+	+	Schemske 1980; Murren & Ellisson 1996
Calopogon tuberosus (L.) Britton, Sterns & Poggenb.	1-10		NS	Firmage and Cole 1988
Comparettia falcata Poepp. & Endl.	1-9	+	+	Rodríguez *et al.* 1992
Cyclopogon cranichoides (Griseb.) Schltr.	8 - > 40		+	Calvo 1990
Dactylorhiza maculata (L.) Soó	Mean 15		NS	Vallius 2000
Elythranthera brunonis (Endl.) A.S. George		NS	NS	Tremblay *et al.* this issue
Psychilis krugii (Bello) Sauleda	1-8	NS	NS	Ackerman 1989
Epidendrum exasperatum Rchb. f.	6-358	+		Calvo 1990
Epipactis helleborine (L.) Crantz	15-30? data absent from paper	+	+	Ehlers, *et al.* 2002, Piper and Waite 1988
Gastrodia exilis Hook. f.	2-13		NS	Pedersen *et al.* 2004
Ionopsis utricularioides (Sw.) Lindl.	1-44	+	+(-)*	Montalvo & Ackerman, 1987
Lepanthes wendlandii Rchb. f.	1-123		NS	Calvo 1990
Malaxis massonii (Ridl.) Kuntze	6-106	+	+ year dependent	Aragón & Ackerman 2001
Oeceoclades maculata (Lindl.) Lindl.	4-16		NS	Calvo 1990
Orchis purpurea Huds.	9-98		NS	Jacquemyn *et al.* 2002
Platanthera bifolia (L.) Rich.	10-20	+	+	Maad 2000
Oligochaetochilus (Pterostylis) longifolia (R. Br.) Szlach.	1-5 data absent from paper	NS	NS	Hamilton 2003
Rhyncholaelia glauca (Lindl.) Schltr.	1-17	NS	NS	Flores-Palacios & Garcia-Franco 2003
Tolumnia variegata (Sw.) Braem	1-15		D	Sabat & Ackerman 1996

* Large inflorescences produce more fruits than small inflorescences, but for those plants that produce fruit, there is a strong *negative* relationship between fruit number and flower number.

morphisms among species with similar pollination strategies suggests that other factors are at play.

However consistency in selection of larger inflorescence appears to be common. Selection on floral display size and reproductive successes clearly shows that larger inflorescences size appears to offer an advantage. However, the observed advantage may not be easily explained. Tremblay (2006) showed that larger display sizes are advantageous in *Caladenia gracilis,* which was not expected, as this species belongs to a mostly single flowered clade of the Caladeniinae. If this evolutionary advantage has been present prior to the study, then why are larger inflorescences not more common in this clade? Constraints to display size and its evolution must be present and could be influenced by complex heritability, inbreeding depression, costs to reproduction and phenotypic plasticity. Behavioral differences among pollinators of the different species may also play a role as would the ecological context in which the orchid populations exist. To evaluate the potential for natural selection on floral display a number of parameters need to be evaluated. Do flowering individuals come back with the same number of flowers (at least in the same range) as previous flowering events? Is the lifetime reproductive success of a large individual equal to small individuals? What is the importance of phenotypic plasticity on floral display? At present those data are generally missing from the literature for terrestrial and epiphytic orchids.

We found no evidence in the orchid literature on the frequency of individuals expressing a specific floral display size among flowering period. Determining if that display size is genetically influenced, however a substantial amount of evidence suggests that there is a cost to reproduction in many species of orchids which often results in reduce display size in the next reproductive bout or emerging as vegetative individual or not at all (dormant) (Pfeifer *et al*. 2006, Coates *et al*. 2006 Tremblay *et al*. 2005). Consequently display size may be very plastic and not a good character for predicting selection coefficients. Short surveys maybe inconsequential to evolutionary processes. It may be more appropriate to evaluate this character considering the lifetime reproductive success of the individuals and a measure of mean display size throughout that lifespan.

Gentry and Dodson (1987) have suggested that evolutionary processes in orchids can be quick and that within a few generations cladogenesis can occur. Under a basic Darwinian evolutionary process selection coefficients would then have to be high and consistent among time periods for evolution to be quick. Others contend that such processes may be slow (Soto-Arenas

1996) and there is some genetic evidence for it (Corrias *et al*. 1991, Rossi *et al*. 1992, Ackerman & Ward 1999). A third possibility is that in many situations, genetic drift may be equally important as natural selection in fostering genetic and morphological variation in this family (Tremblay *et al*. 2005). We favor this vision and suggest that the great diversity in this family to be largely a consequence of sequential and rapid interplay between drift and natural selection.

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