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Variation in floral morph ratios in tristylous *Oxalis squamata* (Oxalidaceae): an Andean alpine endemic

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Abstract: Negative frequency-dependent selection resulting from disassortative mating should result in equal morph ratios in tristylous populations at equilibrium. However, surveys of morph-frequency variation in tristylous species commonly report deviations from equality. Here, we report variation in morph ratios in *Oxalis squamata* Zucc., an endemic of the Andean region of Chile and Argentina. Absence of clonal propagation in this species allows unambiguous estimates of the morph ratio of genets. We sampled floral morph ratios in 20 populations occurring in Central Chile and investigated the relation between morph evenness in populations and their size. All populations of *O. squamata* were tristylous but with significant heterogeneity among populations in morph ratios. Although small populations exhibited a greater variance in morph evenness, biased ratios were also evident in several large populations. We found no evidence of morph loss or a consistent bias in morph frequencies as reported in some tristylous species. Biased morph frequencies in large populations probably arise from episodic sexual recruitment following disturbance and a slow approach to equilibrium.

Key words: Andes, *Oxalis squamata*, nonequilibrium conditions, population size, stochastic forces & tristly.

Résumé : La sélection négative dépendant de la fréquence résultant de croisements désassortis devrait conduire à des ratios de morphes égaux à l'équilibre, chez les populations tristyles. Cependant, les suivis de la variation du ratio des morphes chez les espèces tristyles rapportent généralement des dérivations par rapport à l'égalité. Les auteurs font état de la variation du ratio des morphes chez l'*Oxalis squamata* Zucc., une espèce endémique de la région des Andes, au Chili et en Argentine. L'absence de propagation clonale chez cette espèce permet d'estimer sans ambiguïté le ratio des morphes des genets. Les auteurs ont échantillonné les ratios des morphes florales dans 20 populations venant au centre du Chili et ils ont examiné la relation entre l'égalité des morphes dans les populations et leur étendue. Toutes les populations de l'*O. squamata* étaient tristyles, mais avec un hétérogénéité significative des ratios des morphes entre les populations. Bien que les petites populations montrent une plus grande variance dans les morphes, on a également observé des ratios biaisés dans plusieurs grandes populations. Les auteurs n'ont pas obtenu de preuves de perte de morphes ou un biais cohérent dans les fréquences des morphes, comme on le rapporte chez certaines espèces tristyles. Les fréquences de morphes biaisées dans les grandes populations proviennent probablement d'un recrutement sexuel épisodique suivant une perturbation, avec un lent retour à l'équilibre.

Mots-clés : Andes, *Oxalis squamata*, conditions de nonéquilibre, dimension des populations, forces stochastiques et tristylie.

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Introduction

Populations of tristylous species are characterized by the occurrence of three floral morphs, which differ principally in style length, anther height, and incompatibility relationships (Darwin 1877). The floral polymorphism is maintained in populations by negative frequency-dependent selection result-ing from intermorph (disassortative) mating (Barrett 1993). Mating patterns in populations are controlled by a trimorphic self-incompatibility system that limits self and intramorph mating and promotes fertilizations between anthers and stigmas of equivalent height (Barrett and Cruzan 1994). In large equilibrium populations, 1:1:1 (isoplethic) morph ratios are predicted, as long as there are no fitness differences among the

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floral morphs (Fisher 1944; Spieth and Novitski 1969; Heuch 1979). However, surveys of morph frequencies in tristylous populations often demonstrate deviations from isoplethy (see Fig. 5 in Barrett 1993), raising the question of the ecological and genetic factors that might account for biased (anisoplethic) morph ratios. This issue is of general significance, because it concerns the relative importance of natural selection and genetic drift in maintaining genetic polymorphism.

Two principal evolutionary processes can account for anisoplethic morph ratios in tristylous populations. First, natural selection can lead to biased morph ratios and the loss of floral morphs from populations resulting in stylar dimorphism and monomorphism. A variety of selective mechanisms have been implicated in causing anisoplethic ratios and morph loss, including morph-specific differences in selfing rates (Barrett et al. 1989), variation in pollen production and male fertility (Barrett et al. 1983), asymmetrical pollen transfer and mating (Barrett et al. 2004), and evolutionary modifications to floral morphology and trimorphic incompatibility (Lewis 1975; Weller 1976). Second, stochastic fluctuations in population size, including genetic drift, bottlenecks and founder events, can also result in biased morph ratios and morph loss (Husband and Barrett 1992). Also, many tristylous species are clonal perennials with limited sexual recruitment and biased morph ratios can reflect historical contingencies associated with population establishment followed by slow progress to the isoplethic equilibrium (Ornduff 1972; Barrett and Forno 1982; Morgan and Barrett 1988; Eckert and Barrett 1995). Indeed, although nonequilibrium conditions in clonal plant populations are probably ubiquitous, they are rarely considered when interpreting patterns of variation in sexual polymorphisms (reviewed in Barrett et al. 2010), perhaps owing to the greater historical interest in natural selection.

Tristyly is reliably reported from six flowering plant families (Amaryllidaceae, Connaraceae, Linaceae, Lythraceae, Oxalidaceae, and Pontederiaceae (Barrett 1993; Thompson et al. 1996)). *Oxalis* (Oxalidaceae), a cosmopolitan genus of ~500 species of primarily perennial herbs (Mabberley 1987), contains more tristylous species than all other tristylous taxa combined. Surveys of style morph frequencies in populations of *Oxalis* species commonly report deviations from isoplethy and populations missing floral morphs (reviewed in Ornduff 1972; Weller and Denton 1976; Marco and Arroyo 1998; Turketti 2010). In some cases this appears to result from founder events followed by vegetative propagation, as *Oxalis* species often exhibit a prolific capacity to regenerate by clonal reproduction (e.g., Mulcahy 1964; Ornduff 1972; Luo et al. 2006; Castro et al. 2007; Zeitsman et al. 2008). However, biased morph ratios in *Oxalis* can also arise from natural selection associated with the breakdown of tristyly to distyly, and this often (although not exclusively) involves the loss of the mid-styled morph from populations (Weller 1986; Weller et al. 2007; Pérez-Alquicira et al. 2010). Despite a large literature on tristyly in *Oxalis*, to our knowledge an explicit species-level study of the influence of finite population size on morph-ratio variation has not been conducted. The absence of data on the relation between morph ratios and population size may reflect the fact that in many *Oxalis* species clonal propagation complicates efforts to accurately estimate population size and genet morph ratios.

Here, we investigate variation in floral morph ratios in tristylous *Oxalis squamata* Zucc., a perennial herb endemic to the Andes of Chile and Argentina. We chose this species for the following reasons: (*i*) the species is nonclonal (Marco and Arroyo 1998), and as a result individual genets are easily distinguished under field conditions allowing morph ratios to be determined; (*ii*) individual plants flower throughout the entire summer so that reliable sampling of all flowering plants in a population is possible; (*iii*) in common with several other Andean *Oxalis* species, *O. squamata* has been described as distylous, with only the L and S-morphs (Lourteig 1988). However, a more recent study (Marco and Arroyo 1998) provided evidence of tristyly. Surveys of morph ratios should help to resolve the heterostylous status of *O. squamata*; and (*iv*) our preliminary observations indicated that *O. squamata* populations varied considerably in size and habitat conditions ranging from disturbed roadsides to intact alpine vegetation. Populations were frequent on disturbed sites, where they tended to be denser and often much larger than in intact vegetation. The evident colonizing ability of *O. squamata* and associated ecological and demographic variation has the potential to affect morph ratios through the influence of stochastic processes. Our study addressed the following specific questions: (*i*) How common is isoplethy in *O. squamata* and is there evidence of consistent bias and (or) morph loss among populations? (*ii*) What is the relation between population size and morph evenness? Under a purely stochastic model we would expect small populations to exhibit greater variance in morph ratios whereas larger populations should be closer to the isoplethic equilibrium of 1:1:1.

Materials and methods

Study system and sampling

Oxalis squamata is a tufted perennial herb with numerous attractive pink flowers (Fig. 1). It forms conspicuous populations from 2000 to 3100 m above sea level in the alpine zone of the Chilean Andes (33°S), where our study was conducted. Plants are completely covered in snow throughout the winter period, with flowering occurring from December to February in the austral summer. Pollination is mediated by small bees, mostly Andrenidae and Megachilidae (Marco and Arroyo 1998). Details of the tristylous syndrome of *O. squamata*, including measurements of floral morphology and the results of controlled legitimate and illegitimate pollinations are described in Marco and Arroyo (1998). In brief, the species possesses typical features of the tristylous syndrome, including reciprocal stigma and stamen heights, pollen trimorphism, and a moderately strong trimorphic incompatibility system. In the single large population studied by Marco and Arroyo (1998) near Farellones, Central Chile, floral morph frequencies were close to 1:1:1.

In January 2010, at peak flowering, we sampled 20 populations of *O. squamata* along two large adjacent mountain valleys from Farellones to La Parva and from Farellones to Valle Nevado. The localities and habitats of populations are provided in Table 1. Populations ranged in altitude from 2000 to 3000 m and were separated from one another by at least 2 km and more often 5 km. Most populations occurred within 500 m of roads or mountain tracks, either in intact alpine vegetation, or more commonly in disturbed areas associated with road building. In 13 of the 20 populations, the floral

Fig. 1. A flowering individual of tristylous *Oxalis squamata* near Valle Nevado, Central Chile.

morph identity of all flowering plants was determined by inspection; the remaining seven populations plants were sampled at 2 m intervals along transects running through populations and the style morph of plants recorded. We estimated the size of each population based on the number of flowering individuals. The vast majority of plants (>90%) were reproductive in the populations we sampled.

We used likelihood ratio (*G* tests) tests to explore variation in floral morph ratios and compared their frequencies to isoplethic expectations with heterogeneity *G* tests (Sokal and Rohlf 1995), and we used sequential Bonferroni adjustment to control the overall Type 1 error rate. We investigated the relation between population size (log) and the evenness (*E*) of morphs within population. Following Barrett et al. (1989) evenness was calculated as:

$$E = \frac{1 - (f(L)^2 + f(M)^2 + f(S)^2)}{0.6667}$$

where $f(X) = $ frequency of X-morph.

We also conducted a similar analysis to check if this result was robust, using the Euclidean distance (*O*) of populations from isoplethy following Husband and Barrett (1992, see their fig. 1).

Results

Populations of *O. squamata* ranged in size from 37 to 670 individuals (mean and median population size 280 and 245.5,
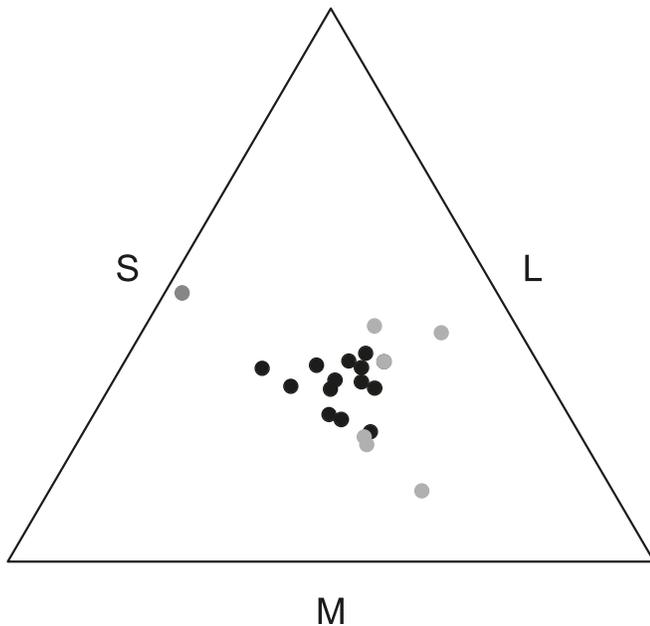
Table 1. The localities, habitat, and floral morph frequencies of 20 *Oxalis squamata* populations sampled in this study.

Population	Location	Habitat	Morph frequency			Sample size (*n*)	Population size
			L	M	S		
1	Curve 6, Valle Nevado	Disturbed roadside	0.28	0.35	0.37	309	500
2*	By Valle Nevado hotel	Embankment	0.24	0.36	0.40	311	311
3	Curve 2, Valle Nevado	Rocky slope alpine vegetation	0.28	0.32	0.41	304	600
4	Curve 8, Valle Nevado	Roadside bank	0.37	0.27	0.36	310	650
5	La Parva west side of creek	Disturbed slope	0.26	0.38	0.36	309	400
6*	El Colorado	Disturbed slope	0.49	0.49	0.02	123	123
7	Between curves 3–4, Valle Nevado	Disturbed roadside	0.35	0.31	0.34	411	500
8*	2 km below Farellones	Disturbed roadside	0.13	0.42	0.47	65	64
9*	Farellones	Roadside bank	0.30	0.13	0.57	54	54
10	Curve 17, Valle Nevado	Roadside bank	0.29	0.33	0.38	670	670
11	La Parva waterfall	Roadside bank	0.32	0.24	0.44	93	93
12	Junction of La Parva and El Colorado Rds	Roadside bank	0.43	0.35	0.22	37	37
13	"Rockhouse" Rd to Valle Nevado	Roadside bank	0.40	0.32	0.28	47	47
14	Curve 1, Valle Nevado	Flat rocky ledge	0.36	0.26	0.39	174	174
15*	Curve 15, Valle Nevado	Waterfall gully	0.22	0.43	0.35	299	299
16	Curve 19, Valle Nevado	Roadside bank	0.29	0.36	0.34	192	192
17*	Lodge Cordillera, La Parva Rd	Under *Senecio* bushes	0.34	0.23	0.44	211	350
18	2 km below La Parva	Steep bank	0.35	0.36	0.30	84	84
19*	La Parva	Steep bank	0.34	0.21	0.45	215	350
20	La Parva	Reforestation plot with birch	0.33	0.33	0.34	106	106

Note: Pop. 2, *G* = 14.01, *P* = 0.0009; Pop. 6, *G* = 75.7, *P* < 0.0001; Pop. 8, *G* = 15.47, *P* = 0.0004; Pop. 9, *G* = 16.71, *P* = 0.0002; Pop. 15, *G* = 20.59, *P* < 0.0001; Pop. 17, *G* = 14.08, *P* = 0.0009; Pop. 19, *G* = 18.03, *P* = 0.0001 using α = 0.05/20 and *df* = 2. Without Bonferroni correction and α = 0.05, four additional populations (3, 4, 5, and 10) would be classed as anisoplethic.

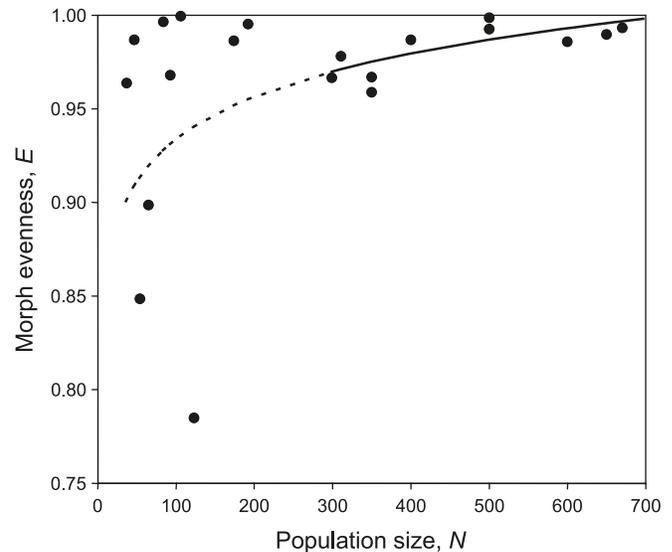*Populations with a significant deviation from isoplethy.

Fig. 2. Floral morph frequencies in 20 populations of *Oxalis squamata* sampled from Central Chile. Points in the triangle represent individual populations with the distance of a point from each axis proportional to the frequency of the floral morph in the population. For example, the point (population 6) close to the S-axis contains a low frequency of the S-morph. Isoplethy is equidistant from each of the three axes and the seven populations with anisoplethic morph ratios are indicated in light grey.

Fig. 3. The relation between population size (*N*) and morph evenness (*E*) among 20 populations of *Oxalis squamata* in Central Chile. A value of 1 for *E* = isoplethy. The equation for this relation is: $E = 0.78 + 0.033* \ln(N)$ for the 10 largest populations (solid line) and the extrapolated range (dotted line) for this relation is also indicated (see Results for further details).

respectively). All populations sampled were tristylous and the overall mean frequency of floral morphs among the total sample was close to isoplethy ($n = 5605$ plants; L-morph = 0.317; M-morph = 0.322; S-morph = 0.361). However, not unexpectedly given the large sample size of plants both the total and pooled *G* values indicated significant deviation from equal morph frequencies ($G_{total} = 227.5$, df = 40; $G_{pooled} = 27.48$, df = 2, both $P < 0.001$). There was considerable variation among populations in the relative frequencies of morphs (Table 1; Fig. 2) and this heterogeneity was statistically significant ($G_{het} = 200.02$, df = 38, $P < 0.001$). Among the 20 populations that we sampled, 7 (35%) exhibited morph ratios that were significantly different from isoplethy after Bonferroni correction.

The relationship between both morph evenness (*E*) and Euclidian distance from isoplethy (*O*) and the natural log of population size was marginally significant $0.05 < P < 0.01$ ($E - F_{1,18} = 3.2$, $P = 0.090$, Fig. 3; $O - F_{1,18} = 3.44$, $P = 0.080$). In both cases the intercepts of these relations were significant ($E - t = 12.36$, $P < 0.001$; $O - t = 2.93$, $P = 0.009$) but the slopes were not using an α = 0.05. However, as is evident from Fig. 3 there is a tendency for greater variation in morph frequencies in smaller than larger populations, as would be predicted based on the effects of finite population size. This trend was supported by the fact that the standard deviation of morph evenness for the 10 smallest populations (SD = 0.074) was 5.5 times larger than the standard deviation of the 10 largest populations (SD = 0.013). This disparity was significantly different following Levene's test ($F_{1,18} = 14.09$,

$P = 0.002$), whereas the mean *E* values for the 10 largest and 10 smallest populations were not significantly different (Welch's Test for equality of means ($F_{1,10} = 2.67$, $P = 0.14$). The same result was obtained in the comparison of standard deviations using the Euclidian distance from isoplethy (results not shown). Given the large variation in morph evenness in the 10 smallest populations, we tested for a relation between both morph evenness (*E*) and log population size in the 10 largest populations. The test was significant ($E - F_{1,8} = 10.8$, $P = 0.011$; intercept = 0.779, $t = 12.57$, $P < 0.0001$; ln(pop size) = 0.0334, $t = 3.29$, $P = 0.011$). The predicted line from the model is illustrated in Fig. 3 (solid line based on 10 largest populations and dashed line for the extrapolated range).

Discussion

Our survey revealed significant heterogeneity in floral morph frequencies among population of *O. squamata* in the Andes of Central Chile (Fig. 2). Although the mean morph ratio across the complete sample of 20 populations was close to isoplethy, 35% of the populations exhibited skewed ratios and these were not necessarily restricted to the smallest populations in our sample. Below we discuss the ecological and genetic mechanisms that are likely responsible for the patterns that we observed and consider more generally the maintenance and breakdown of tristyly in *Oxalis*.

In contrast to surveys of many *Oxalis* species, we found no morph loss from populations of *O. squamata*. Thus, there is no evidence that in this species distyly is likely to evolve from tristyly, at least in the region we studied. Among the populations surveyed, only one was characterized by a very low frequency of the minority morph and this was of moderate size (population 6: $n = 123$ plants; S-morph ~0.024%). Theoretical studies indicate that species with trimorphic incompatibil-

ity are quite resilient to stochastic morph loss and can maintain tristyly as long as population sizes are not less than 20 individuals (Heuch 1980). None of the populations we encountered in our survey were this small. Moreover, *O. squamata* is not rare in the area studied and gene flow among populations may also serve to maintain tristyly, if populations are occasionally small enough to lose morphs through drift. Elsewhere, gene flow has been invoked to explain the maintenance of tristyly in small populations of *Lythrum salicaria* (Halkka and Halkka 1974; Eckert et al. 1996*a*). Unfortunately, knowledge of the mechanisms and extent of gene flow among plant populations in most alpine environments is poorly understood.

As predicted, we observed more variation in morph frequencies in small than large populations of *O. squamata* (Fig. 3). This pattern has been observed in populations of other tristylous species (e.g., Husband and Barrett 1992; Eckert and Barrett 1992) and is undoubtedly associated with the greater likelihood of random sampling effects in small populations. However, several of the larger populations we sampled were also characterized by uneven morph frequencies (e.g., populations 2, 3, 4, 5, 10, 17, 19 see Table 1). Because these populations all contained >300 individuals, it is worth considering why morph frequencies were not closer to the expected isoplethic equilibrium.

Experimental studies of *O. squamata* have provided no evidence that morph-specific fitness differences might explain these patterns. Controlled crosses and measurements of open-pollinated seed production indicate that the floral morphs have equivalent fertility, and unlike other *Oxalis* species in which biased morph ratios are associated with selection (e.g., *Oxalis alpina*; Weller 1986; Weller et al. 2007), the morphologies of the floral morphs show no sign of evolutionary modifications (Marco and Arroyo 1998). Thus, because there was no consistent bias in favour or against any particular style morph it seems more likely that the anisoplethic morph ratios we observed are largely the result of stochastic processes.

The inheritance of tristyly is unknown in *O. squamata.* However, studies of other *Oxalis* species, including the sister species *O. valdiviensis* (Fyfe 1950; Heibl and Renner 2012) and members of other tristylous families, indicate that two epistatically interacting diallelic loci (*S* and *M*) govern the expression of the polymorphism (reviewed in Lewis and Jones 1992; Barrett 1993). Heuch (1979) demonstrated that isoplethy is a necessary equilibrium condition regardless of the genetic model for tristyly (and see Heuch and Lie 1985). Thus, we assume that 1:1:1 morph frequencies, as we observed in some populations, should be attainable in large equilibrium populations as a result of disassortative mating.

The most likely explanation as to why in some populations this was not the case relates to the assumption that large populations are necessarily closer to the isoplethic equilibrium. Although in general this may often be true, aspects of the ecology of *O. squamata* make this inference less certain. In tristylous populations the approach to equilibrium can be quite protracted, especially when episodic sexual recruitment occurs (Eckert and Barrett 1995, Eckert et al. 1996*b*). In *O. squamata* this seems probable as many of the populations we surveyed along roadsides appeared to be relatively recent in origin, and human disturbance associated with road building and development may have promoted initial bursts of recruitment. The small seeds of *O. squamata* are readily transported downhill

along roadsides by annual snowmelt events in the early spring providing a source of colonists for these sites. Episodic recruitment combined with founder events and a slow approach to isoplethy may explain the biased morph ratios we observed in 35% of the populations we surveyed.

Theoretical studies on the influence of population establishment on morph ratio dynamics indicate that nonequilibrium morph ratios are strongly influenced by the composition of founding genotypes. Significantly, nonequilibrium tristylous populations tend to be characterized by an excess of the S-morph and a deficiency of the L-morph (see Fig. 3 of Morgan and Barrett 1988). This pattern arises because allele frequencies at the *S* and *M* loci approach the equilibrium asymptotically and the S-morph must be represented among the founding individuals for a trimorphic population to establish. Thus, the biased morph ratios that we observed in several large populations of *O. squamata* probably reflect the historical signature of founding genotypes and their subsequent mating patterns. Unfortunately, our sample of 20 populations was too limited to test this hypothesis rigorously, although it is interesting to note that the S-morph was the most frequent morph in 11 out of the 20 populations we sampled. Large-scale sampling of numerous tristylous populations would be necessary to detect historical contingency imposed by the genetics of tristyly (e.g., Morgan and Barrett 1988; Barrett et al. 1989; Eckert and Barrett 1992). Studies of this type combined with demographic information can provide valuable insights into the evolutionary processes governing morph ratios in tristylous populations.

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