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Patterns of phenotypic selection for oil and nectar in *Monttea aphylla* (Plantaginaceae) in a geographic mosaic of interactions with pollinators

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ABSTRACT

Oil-flower species are highly specialized attracting a narrow group of oil-collecting bees in contrast to nectar-flower species visited by a wide diversity of pollinators. The vast majority of the oil species lack nectar as a pollinator reward; this suggests that the ancestors of plants with oil flowers had either nectar-less flowers or faced strong selection against the production of nectar once oil secretion evolved. *Monttea aphylla* is one of the few species that simultaneously offers oil and nectar in the same flower to pollinators. In particular, we studied phenotypic selection in 16 populations in the Monte desert of Argentina that spanned the entire geographical range of the species. In each population, we determined the relationship between plant pollination success and floral rewards. Positive directional selection differentials were detected in three of the populations. One northern population favoured selection on oil production, two central or southern populations favoured selection on nectar production. Directional selection gradients were consistent with the above results and correlational selection was significant for one northern population where pollination success was favoured by an increased selection on oil and a decreased selection on nectar. Geographic variation in phenotypic selection models showed latitudinal increase in directional selection on nectar. Geographic variation in visitation frequencies of the specialist oil-collecting bee and relative abundance of other floral sources were related to an increase in directional selection on nectar. Thus, evolution of rewards appears to respond to a geographic mosaic where either oil or nectar are favoured in different contexts of ecologically specialised oil collecting bees. Although phenotypic selection was detectable in few and was absent in most populations, the general pattern was consistent with current geographic differentiation in reward amounts.

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1. Introduction

Remarkable diversity in flower phenotype has been taken as evidence of the important role of pollinator mediated natural selection in the evolution and diversification of angiosperm flowers (Darwin, 1859; Conner et al., 1996). The role of pollinators for promoting the evolution of phenotypic novelties is of great interest in the study of the evolutionary ecology of plant-pollinator interactions (Stebbins, 1970; Faegri and van der Pijl, 1979; Herrera and Pellmyr, 2002; Fenster et al., 2004; Harder and Johnson, 2009). Unusual or novel rewards that are collected by a single functional group of pollinators are specially interesting, because they can promote diversification through specialized plant-pollinator interactions.

Stefan Vogel discovered fatty oils as reward that mediates specialized pollination interactions with female oil-collecting bees and explored in great detail the biology of both the plants that produce this reward and the bees that collect them (Vogel, 1971, 1974, 1976, 1981, 1986, 1988, 1990). Floral oil as a reward for pollinators has evolved at least 28 times among angiosperm families in tropical and temperate ecosystems (Vogel, 1974; Buchmann, 1987; Renner and Schaefer, 2010; see Neff and Simpson, 2017). The vast majority of species with oil flowers lack nectar as a reward to pollinators (Vogel, 1974; Simpson et al., 1990; Steiner and Whitehead, 1991). This suggests that the ancestors of plants with oil flowers were either nectar-less or nectar was strongly selected against in favour of oil. If both rewards can coexist, even briefly over evolutionary time, it is of considerable interest, because it may provide valuable insights into the ecological factors necessary for the evolution of this relatively uncommon type of floral reward. Microevolutionary processes are more appropriately studied at the level of multiple

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local populations, each of which is subjected to a unique evolutionary trajectory when responding to local selective pressures (eg. [Cosacov et al., 2014](#)). A species may, thus, be viewed as a collection of evolutionary experiments ([Thompson, 2005](#)).

Given a geographic backdrop of variable biotic regimes, the magnitude and mode of selection on floral traits may vary across the distribution range of a species ([Gilbert et al., 1996](#); [Herrera et al., 2006](#); [Rey et al., 2006](#); [Nattero et al., 2010a,b](#); [Nagano et al., 2014](#)). Consequently, differences, both in composition and relative abundance of pollinators among populations could potentially account for local adaptations depending on the particular ecological context of each population. Moreover, the coexistence of other plant species in the community may affect the strength and/or direction of phenotypic selection on floral traits mediated by pollinators ([Caruso, 2000, 2001](#)). Populations are also expected to reach evolutionary stasis when there are no individual differences in fitness or variation in the focal trait is much reduced due to prolonged selection ([Conner et al., 1996](#); [Herrera, 1996](#); [Benitez-Vieyra et al., 2012](#)).

By explicitly including geographic variation in the study of plant-animal interactions it should be possible to evaluate the formation of pollination ecotypes as a result of geographically varying pollinator mediated selection ([Herrera et al., 2006](#); [Johnson, 2006](#); [Rey et al., 2009](#); [Cosacov et al., 2014](#); [Newman et al., 2014](#); [van der Niet et al., 2014](#)). Thus, the evolution of a given trait or set of traits can only be understood by studying interactions in multiple localities across the range of a species. However, most of the available evidence on phenotypic selection on flower traits comes from single-population studies and a small part explicitly addresses the premises of selection in geographic mosaics along the whole range of a species (see [Caruso, 2001](#); [Totland, 2001](#); [Herrera et al., 2006](#); [Rey et al., 2006](#); [Harder and Johnson, 2009](#); [Zhao and Wang, 2015](#)). To our knowledge, studies specifically addressing selection on flower rewards have not included spatial replicates ([Gleiser et al., 2014](#); [Gijbels et al., 2015](#); but see [Paiaro, 2011](#)). In addition, the analysis of co-flowering plant community and its relationship with phenotypic selection mediated by pollinators has not been evaluated in a geographical context.

Monttea aphylla is one of the few angiosperms to attract pollinators by simultaneously offering both oil and nectar in the same flower ([Sérsic and Cocucci, 1999](#)). In addition, it exhibits a marked latitudinal pattern in the amount of both rewards, with oil volume increasing and nectar decreasing towards the tropics ([Fig. 1a](#); [Ferreiro et al., 2015](#)). *Monttea aphylla* is essentially pollinated by assemblages consisting of female bees of the genus *Centris* which collect oil and nectar ([Sérsic and Cocucci, 1999](#); [Ferreiro et al., 2015](#); [Fig. 1b](#) and [c](#)). The assemblages differ markedly in their ecological specialization, from the extreme specialist, *Centris vardyorum*, known to utilise only *M. aphylla* for its oil and nectar supply, and two additional generalist *Centris* species (*C. tricolor* and *C. brethesi*), which forage from a wide range of oil and nectar sources ([Michelette and Camargo, 2000](#); [Tadey, 2011](#); [Chacoff et al., 2012](#)). It is relevant from a geographic context that the *M. aphylla* specialist *C. vardyorum* exhibited a marked latitudinal variation in abundance, being the dominant pollinator mostly in southern temperate areas ([Fig. 1a](#)), whereas the generalist *Centris* species were relatively more abundant in the tropical areas to the north. This geographic setting has the potential to create geographically divergent selection patterns on floral rewards within *M. aphylla*. According to this background knowledge on this plant-pollinator system we can hypothesize that the large-scale geographical variability in reward quantity is promoted by different local optima throughout the species range, related to processes that operate in biotic (pollinators and co-occurring plant species) contexts.

In the present study we explore the patterns of phenotypic selection on floral rewards across a broad geographical scale, to answer the following specific questions: (1) Are nectar and oil

rewards subjected to pollinator mediated phenotypic selection?, (2) If phenotypic selection on floral rewards is detected, is there any geographical pattern in the magnitude and direction of selection across the species distribution range?, (3) Are these patterns associated with variation in pollinator assemblages and co-flowering plants?

2. Materials and methods

2.1. Plant species and study sites

Monttea aphylla (Plantaginaceae) is a leafless, up to 2 m high shrub endemic of the Monte Desert of Argentina ([Rossow, 1985](#)). Plants bearing 20–100 axillary, violet, tubular and hermaphroditic flowers ([Tadey 2011](#)). The oil-secreting area of the flower, the elaiophore, is located in a simple pouch-like depression in the ventral portion of the corolla, and consists of trichomatic oil-producing glands which roughly form a triangular and continuous patch ([Ferreiro et al., 2015](#)). Nectar is located at the base of the corolla tube, produced by a thick-lobed ring nectary between the ovary and the non-glandular corolla ([Ferreiro et al., 2015](#)). Fruit set due to self-pollination is null or minimal whereas pollen limitation and pollinator dependence has been locally recorded ([Tadey, 2011](#); [Simpson et al., 1990](#)).

Phenotypic selection analysis were performed in 16 populations during flowering season from October to December 2009–2011 ([Fig. 1a](#)). These sites are located approximately between 26°S and 41°S and between 64°W and 69°W, at elevations ranging from 86 to 2531 m ([Table 1](#)). The sampling area covered a 1671 km north–south extension.

2.2. Floral rewards

In each population, 20 flowering individuals and three virgin flowers per individual were collected at random at around 8:00 a.m. to measure the nectar and oil volume (total flowers per site = 60, total measured flowers = 960). For each flower nectar was measured using 1- μ L capillary tubes (Drummond microcaps) and oil volume was measured by gently pressing the elaiophore (oil-secreting region) of the corolla against cigarette rolling paper until the paper was saturated. After allowing the paper to dry for at least 24 h, oil stains on the paper were photographed against a scale, and the volume was estimated using a calibration curve, obtained from castor oil applied to the same type of cigarette paper. Castor oil was chosen for its chemical and physical similarities to floral oil ([Vogel, 1974](#); [Seipold, 2004](#); see [Ferreiro et al., 2015](#)). Previous studies have shown that variation in both rewards was significant at the among individual and population levels ([Ferreiro et al., 2015](#)).

2.3. Pollination success

To measure pollination success, three flowers at post-floral stage were harvested from 20 individuals in each population (total N = 960). The stigmas from these flowers were used to estimate individual pollination success counting the number of germinated pollen tubes on each stigma as a measure of the quantity and quality of pollen receipt. Pollen tube growth was used as a reliable surrogate of pollination success because it correlates with the number of pollen grains deposited on the stigma by pollinators and with other measures of maternal fitness (see [Snow and Roubik, 1987](#); [Aizen and Feisinger, 1994](#); [Herrera et al., 2006](#); [Aizen and Harder, 2007](#)). In a preliminary analysis performed with fruit set obtained from five populations it was observed that the minimum number of fruits produced per inflorescence was significantly associated with the number of pollen tubes per stigma (see [Appendix A](#)). Producing high quality embryos is probably associated to the number

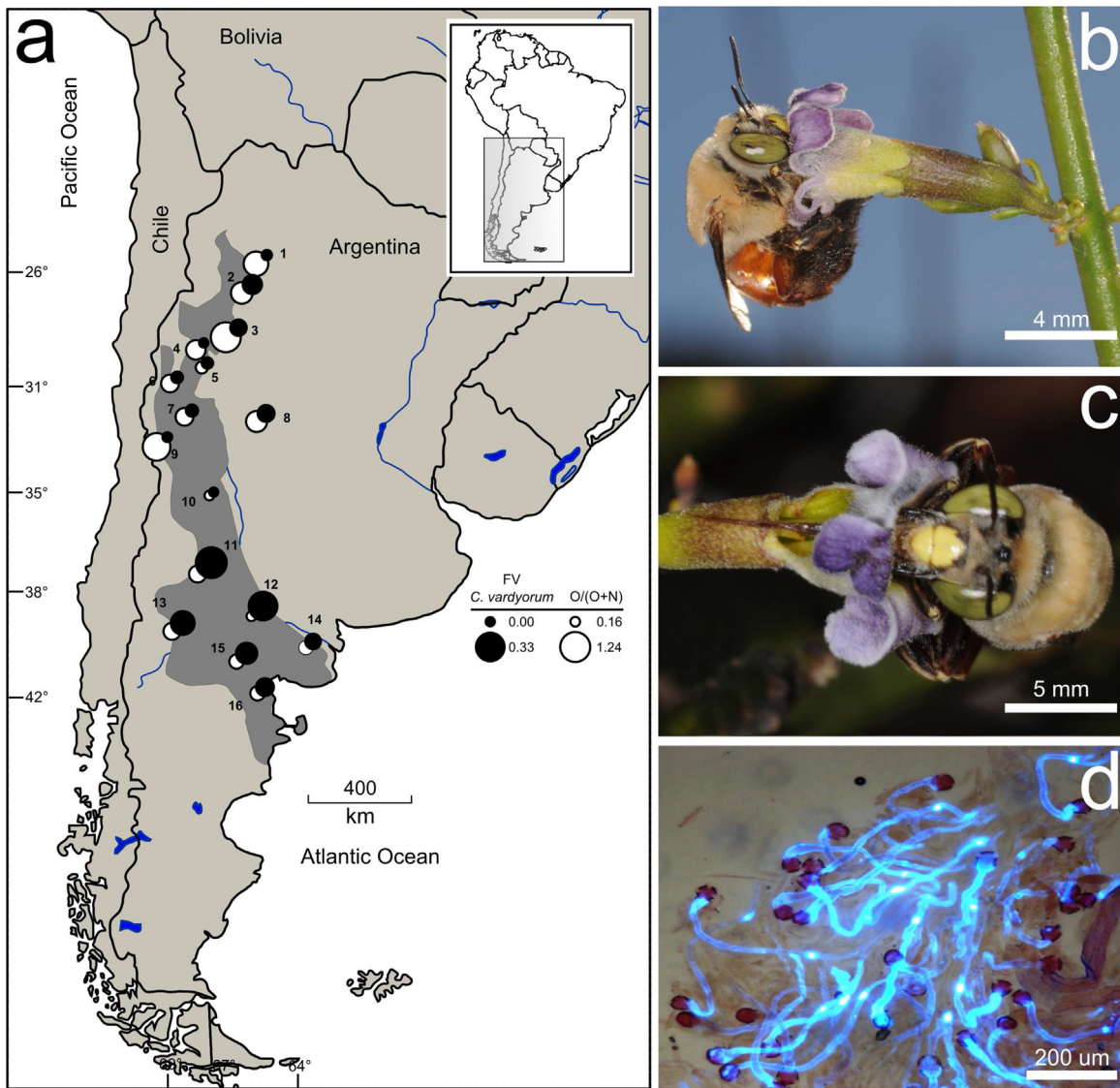


Fig. 1. (a) Geographical variation in the proportion of reward volumes (oil/oil+nectar, white circles) and visitation frequency (VF) of females of *C. vardyorum* (black circles) in 16 populations of *M. aphylla* throughout its geographic range in the Monte desert (Argentina); (b) Female of *Centris brethesi*, collecting floral oil in *Monttea aphylla*; (c) *Centris brethesi* female visiting a damaged flower, note the extended tongue directed towards the base of the floral tube searching for nectar; (d) Pollen tubes germinated on a *M. aphylla* stigma. The diameter of the circles shown in the map is proportional to the corresponding mean value of each trait at each population.

Table 1

Location, floral reward traits (mean and coefficient of variation) and fitness variable (mean and opportunity for selection) of each *M. aphylla* population studied. Populations are ordered by increasing latitude. Sample locations are numbered consecutively (Nloc), as shown on the map in Fig. 1 CV: Coefficient of variation. I: Opportunity for selection.

Nloc	Pop. code	Site	Geographical data			Floral rewards volume average (CV)		Fitness average (I)
			Latitude	Longitude	Altitude	Oil volume	Nectar volume	Pollen tubes
1	AMP	Ampimpa	26.61	65.84	2353	0.79 (49.64)	0.40 (113.36)	49.48 (0.36)
2	BAL	B. Larga	26.97	66.74	2531	0.83 (34.37)	0.22 (96.59)	37.45 (0.28)
3	CUM	C. Miranda	29.35	67.78	2102	1.21 (29.49)	0.51 (67.63)	21.92 (0.30)
4	TAM	Tambillos	29.43	67.86	1559	0.51 (37.04)	0.78 (46.73)	38.58 (0.84)
5	CHI	El Chiflón	30.21	67.56	1179	0.33 (36.04)	0.32 (66.88)	20.77 (1.22)
6	HUA	Hualilán	30.74	68.96	1960	0.49 (33.36)	0.32 (74.33)	29.95 (0.32)
7	EMA	Maradona	31.77	68.79	1332	0.62 (42.97)	0.50 (54.97)	8.9 (0.87)
8	CAD	Cadillo	31.78	65.19	709	0.71 (27.09)	0.25 (96.93)	45.33 (0.35)
9	POT	Potreriillos	32.93	69.23	1567	1.06 (47.68)	0.18 (73.46)	21.9 (0.68)
10	GAL	Gral. Alvear	35.24	67.67	435	0.20 (28.40)	0.09 (73.9)	59.57 (0.29)
11	25M	25 de Mayo	37.5	67.71	420	0.46 (37.09)	0.28 (78.98)	58.53 (0.30)
12	LIC	Lihuel Calel	38.08	65.69	288	0.17 (28.24)	0.44 (39.88)	64.57 (0.18)
13	CUT	Cutralcó	39.04	68.71	343	0.50 (35.61)	0.70 (24.81)	74.97 (0.23)
14	CON	Conesa	40.02	64.44	86	0.53 (31.12)	1.24 (22.67)	18.25 (1.11)
15	VAL	Valcheta	40.64	66.25	213	0.36 (36.99)	0.77 (28.29)	46.64 (0.33)
16	SAG	S. Grande	41.65	65.33	270	0.42 (63.92)	1.25 (57.11)	58.21 (0.22)

of pollen tubes growing on the style since the chances of xenogamous pollination is then higher. Likewise, we should expect that the measure pollination success (pollen tubes) will be correlated with pollen removal, i.e. individuals that are more successful in receiving pollen are also those which are more successful in removing it (e.g. Conner et al., 1995; Morse and Fritz, 1985; Kudo, 2003; Baranzelli et al., 2014). To quantify the total number of pollen tubes per flower, pistils were double stained with fuchsin solution and aniline blue (Dafni, 1992), and then photographed with a Zeiss Axiophot microscope (Karl Zeiss, Oberkochen, Germany; Fig. 1d). Pollen tubes were counted on the images.

2.4. Pollinators and plant community. Methods used to determine pollinator assemblage composition and visitation frequencies (VF) has been already published elsewhere (Ferreiro et al., 2015). The following five pollinator categories were considered: (1) VF of all *Centris* females; (2) VF of *C. tricolor* females; (3) VF of *C. brethesi* females; (4) VF of *C. vardyorum* females; (5) VF of all pollinators other than *C. vardyorum* female bees.

To determine additional floral sources in each community, relative abundance (RA) of oil and nectar plant species were quantified along two 2 × 50 m transects. The following two co-flowering categories were considered: (1) RA of all oil plants, (2) RA of all nectar plants and (3) RA of *M. aphylla*. Plant species vouchers are deposited in CORD.

2.4. Statistical analysis

2.4.1. Phenotypic selection

The opportunity for selection (I), which represents the total potential of selection, i.e. an inference of how strong selection can potentially be, was estimated for each population (Arnold and Wade, 1984; Brodie et al., 1995). Variation in reward traits was measured as the coefficient of variation (CV).

Separate phenotypic selection analyses were performed for each population, using as predicting variables average oil and nectar volume and as response variable pollination success, estimated as the cumulative number of pollen tubes of three flowers per individual. Since less than three flowers were available for few individuals the number of flowers was used as offset to model a standardized rate of pollen tubes observed per flower (Dobson, 1990). Before selection analyses, floral reward variables (x) were standardised ($z = (x - (x \text{ mean})) / (SD)$). To estimate selection differentials and gradients, pollination success of each flower was relativized to the population mean. Linear and quadratic selection differentials (S_i and C_i , respectively) and linear and correlational selection gradients (β_i and γ_{ij} , respectively) were estimated applying standard procedures (Lande and Arnold, 1983; Brodie et al., 1995). Selection differentials estimate total change in the mean reward amount within a generation while selection gradients estimate partial change in mean amount of one reward, i.e. after removing indirect effects of the other reward. The statistical significance (p) of regressions was obtained with generalized linear models (glm). Since the pollination success measure involves counts and because of the degree of overdispersion, error distributions and link functions were *quasi-poisson* and *log link*, respectively.

For traits that were significantly affected by phenotypic selection, we performed non-parametric regressions using cubic spline to depict univariate associations with pollination success. The *gam* routine of *mgcv* package of R statistical software v. 3.0.3 (R Development Core Team, 2014) was used to estimate the cubic splines (Wood, 2006). Smoothing parameters were obtained by minimizing the generalized cross-validation scores (Wood, 2008), and Bayesian standard errors were obtained according to Wood (2006). Finally, we visualized correlational selection acting on two traits plotting pollination success surfaces using the correlational

gradient with the corresponding parametric function of the Lande and Arnold (1983) model as implemented in R.

2.4.2. Geographic variation in phenotypic selection

To evaluate the geographic variation in phenotypic selection on reward quantity, we tested the homogeneity of slopes of the relationship between relative pollination success (pollen tube growth on styles relativized to the respective population) and standardized reward amount across geographic variables. This was analysed using a model on the whole data set where reward quantity and a geographic (latitude) or ecological variable (pollinators and plant community) were used as predictors and pollination success as response variable. The interaction term of this model tests for differences in slopes among populations, and estimates whether the strength of selection on the trait is significantly affected by the geographic or ecological variable (see Strauss et al., 2005; Herrera et al., 2006). Since, populations were considered independent evolutionary units, results derived from this analysis should await confirmation through phylogenetic correction once results of ongoing phylogeographic studies are available (Baranzelli et al., in review).

3. Results

3.1. Phenotypic selection on floral rewards

The opportunity for selection (I) showed population variation, while both reward volumes exhibited high coefficients of variation (Table 1).

For the single population analyses, significant positive directional selection differentials (S_i) on reward volume were detected in three populations where either increased oil (“Ampimpa”; Fig. 2) or increased nectar production (“Cadillo” and “Conesa”; Fig. 2) were selected. That is, oil was selected in the northernmost part of the species range while nectar was selected at the central and southernmost part of the range. Disruptive selection (C_{ii}) was found in “Ampimpa”, where a significant differential was detected on oil, and in “25 de Mayo” and “Potrerillos”, where marginally significant differentials were detected on nectar. Stabilizing selection was found in “Hualilán”, where the differential on oil was marginally significant, and “Gral. Alvear” where the differential on nectar was marginally significant. In both cases, according to the outlook of the cubic spline, the peak of the fitted curve lies within the range of the respective reward, reaching the maximum value around the mean. However, most populations had non-significant differentials across the geographic range (Appendix B).

The multivariate selection analyses were consistent with the univariate analyses with regard to the linear gradients (Appendix C). This means that directional selection on one floral reward was still detectable after removing indirect effects of the other reward. Correlational selection on oil volume and nectar volume was significant only in “Ampimpa” population (Appendix C) where individuals with high oil amounts and low nectar amounts were favoured (Fig. 3).

3.2. Geographic variation in phenotypic selection associated with biotic context

The model with reward quantity and latitude as predictor variables and pollination success as response variable with the whole data set showed that, only for nectar, relationships between reward quantity and pollination success varied significantly and positively across latitude. This means that there was latitudinal variation in directional selection for this reward (Table 2).

A model as above where latitude is replaced with visitation frequencies as the second predictor variable showed that only

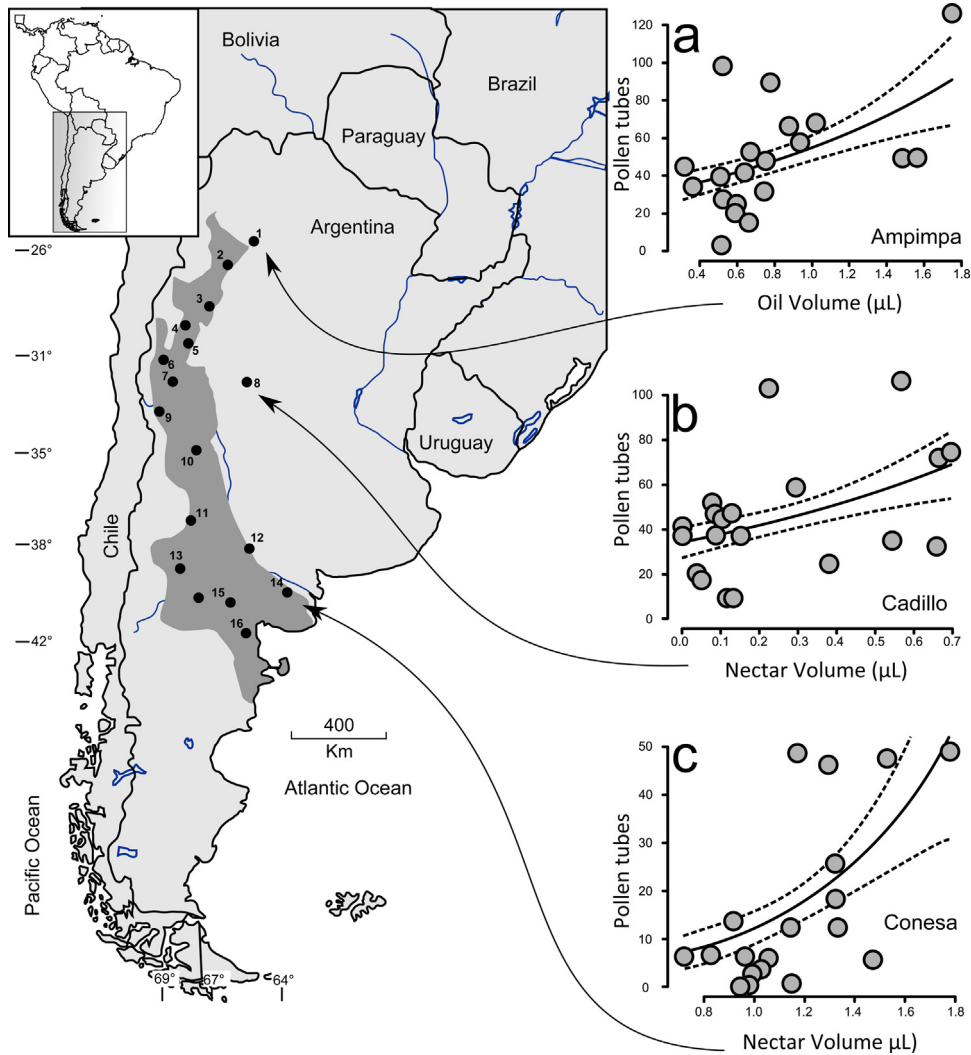


Fig. 2. Cubic spline regressions between standardised floral rewards in *M. aphylla* populations and pollination success (pollen tubes). Dashed lines, ± 1 SD, estimated from ± 1 Bayesian standard errors. (a) Positive directional selection differential on oil volume in AMP population; (b and c) Positive directional selection differentials on nectar volume in CAD and CON populations, respectively. * $P < 0.05$.

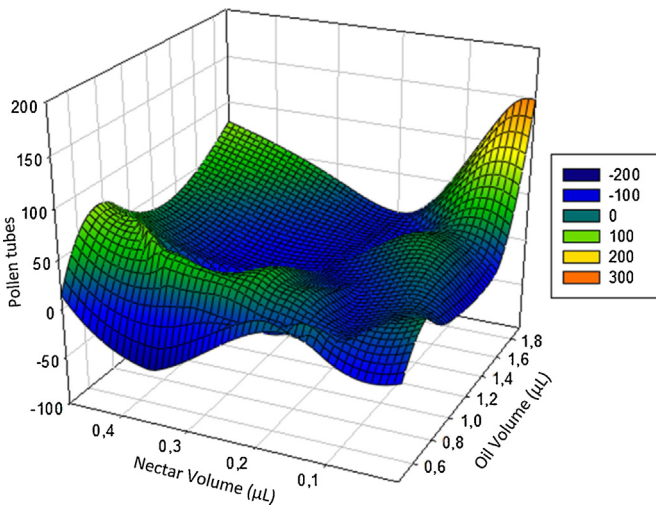


Fig. 3. Selection surface between rewards and pollen tubes as fitness measure in Ampimpa population; γ_{ij} (EE) = -0.54 (0.245), * $P < 0.05$.

Table 2

Homogeneity of slopes test of the relationship between relative pollination success and standardized reward amount (oil or nectar) across latitude, pollinator visitation frequencies assemblages and plant community variables.

Reward	Geographic/ecological Factors	γ_i	\pm (SD)
Oil volume	Latitude	0.000	(0.001)
	VF♀ <i>Centris</i> spp.	-1.388	(1.71)
	VF♀ <i>C.vardyorum</i>	-0.562	(4.13)
	VF♀ <i>C.tricolor</i>	-6.325	(14.445)
	VF♀ <i>C.brethesi</i>	-1.881	(2.166)
	VF non- <i>C.vardyorum</i>	-1.961	(2.134)
	RA oil plants	0.046	(1.141)
	RA nectar plants	-0.010	(0.078)
	RA <i>M. aphylla</i>	0.137	(0.134)
	Nectar volume	Latitude	0.004
VF♀ <i>Centris</i> spp.		2.093	(1.66)
VF♀ <i>C.vardyorum</i>		8.721	(3.949)*
VF♀ <i>C.tricolor</i>		14.846	(13.407)
VF♀ <i>C.brethesi</i>		0.761	(2.019)
VF non- <i>C.vardyorum</i>		1.055	(1.987)
RA oil plants		0.542	(1.165)
RA nectar plants		0.209	(0.076)**
RA <i>M. aphylla</i>		0.217	(0.138)

visitation frequencies of *C. vardyorum* significantly and positively explained the relationship between nectar quantity and pollination success (Table 2); this means that this bee species accounted for a geographical increase in directional selection on nectar. Models including relative abundance of plant species as predictor variables showed that the relationship between nectar and pollination success was significantly and positively explained by the relative abundance of nectar plants. No model with oil quantity as one of the predictor variables was significant in these analyses (Table 2).

4. Discussion

4.1. Phenotypic selection in a geographical context

Both oil and nectar amount per flower were targets of phenotypic selection in some populations suggesting that geographic variation in ecological context imposes evolutionary differentiation among populations. Different rewards had significant directional selection differentials and gradients at opposite extremes of the distribution range. In addition, two populations located at mid-latitudes, which produced relatively low quantities of both rewards (Ferreiro et al., 2015), showed stabilizing selection on oil or nectar. This is consistent with a more broad geographic pattern for nectar since slopes of pollination success on this reward significantly increase to the south. In turn, this agrees with the clinal differentiation in floral rewards, i.e. higher nectar content towards the south reported for the same set of populations (Ferreiro et al., 2015).

Most studies on phenotypic selection on floral traits in changing geographic contexts strengthen the importance of pollinators as drivers of selection on several floral traits (Gómez and Zamora, 2000; Herrera et al., 2006; Rey et al., 2006; Gómez et al., 2009; Nagano et al., 2014), however, very few have explored phenotypic selection on floral rewards with geographic replicates (but see Paiaro, 2011). In addition, to the best of our knowledge, no study has yet found that phenotypic selection patterns are consistent with the floral phenotypic differentiation across the entire species range. Thus, lending support to the idea that phenotypic selection on floral traits may promote differentiation of pollination ecotypes.

4.2. Association between selective patterns for floral rewards and different selective regimes

During the flowering season, *Monttea aphylla* was the only or overwhelmingly predominant oil source at every population. Since oil-collecting bees depend on plants for oil, visitation frequency on *Monttea* would be a reliable proxy for the actual local abundance of oil collecting bees. It can then be said that pollinators of *M. aphylla*, particularly the ecologically specialized *Centris vardyorum* differed in relative abundance across the Monte region. The visitation frequency of this bee is positively correlated with latitude suggesting that it should impose greater selection on floral rewards away from the tropics (Ferreiro et al., 2015). Similarly, the relative abundance of other potential food sources for *M. aphylla* pollinators also exhibited population variation that could potentially influence pollinator mediated phenotypic selection on rewards through interspecific competition (Caruso, 2000; Moeller, 2005; Ferreiro et al., 2015).

In a geographical context, one might expect, following Stebbins (1970), that, where specialized oil-collecting bees are most abundant, there should be greater selection for oil production relative to nectar in *M. aphylla* flowers. Furthermore, assuming that all other oil producing plants are equally attractive, the specialized reward should be favoured over nectar in communities where other oil producing plants are present, due to interspecific competition for the oil-collecting pollination service. Contrarily to these expect-

tations, phenotypic selection for oil production occurred at the northernmost population where the specialized oil-collecting bee was not dominant, where generalist oil-collecting bees acted as pollinators and other oil-flowering species were absent. This result rejects that interspecific competition among oil flowering species for pollinators explains the selection patterns found for oils. Probably intraspecific competition for the scarce pollination services of the ecologically specialized *C. vardyorum* is more important than interspecific competition in this community.

Contrastingly, significant directional selection on nectar was detected in a southern population where the specialized bee was predominant and had a high visitation frequency. Indeed, visitation frequency of *C. vardyorum* significantly explained differences in slopes of the relationship between pollination success and nectar across populations. In those populations where *C. vardyorum* was a mainly pollinator, nectar may be more limiting for the plant reproductive success and therefore there is selection for increased nectar production. Despite also nectar plants were significantly associated to the geographical variation in selective patterns on nectar, due to the high ecological specialization of *C. vardyorum* on *M. aphylla*, interspecific competition among nectar flowering species for pollinators could not explain the pattern found.

Phenotypic selection patterns at a geographic scale are consistent with current geographic differentiation of *M. aphylla* across its entire distribution range. This suggests that current geographic differentiation of floral phenotype could be the result of past phenotypic selection acting in the same direction as in the present. However, selection with the same mode and direction has probably not remained constant in the same populations continuously, but rather hotspots are expected to have shifted locations among different neighbouring populations of the same geographic section. Several studies across the entire range of a species have shown marked variation of functionally significant floral traits corresponding to the geographically variable traits of their pollinators (Anderson and Johnson, 2008; Pauw et al., 2009; Nattero et al., 2011; Cosacov et al., 2014). Present results suggest that only in few hotspots across the underlying geographic backdrop with local and geographically consistent phenotypic selection should be expected. Stasis in the remaining populations (coldspots) is expected to be explained by a number of circumstances such as relaxation of selection due to lack of pollen limitation (that could reduce the opportunity of selection) or to the erosion of phenotypic variability resulting from prolonged stabilizing or directional selection (Herrera, 1996; Conner et al., 1996; Benitez-Vieyra et al., 2012).

The present case is also illustrative of the conditions that could eventually lead to evolutionary divergence in floral rewards. Let us suppose that gene flow between two N-S sections of the current distribution of *Monttea aphylla* was completely interrupted and that current selection patterns were consistent with past selection gradients, then divergent evolution within the species could lead to two clades markedly contrasting in their strategies to attract pollinators. The one clade with dual rewards representing the southern temperate section, and the other clade representing the northern subtropical section with only oil as reward. As a matter of fact, this tendency is currently evident since some individuals in the northern populations contain almost no nectar (Ferreiro et al., 2015). In addition, the correlational selection gradient shows that nectar amount is selected against when oil is favoured in one northern population.

The ecological context within which this proposed pollinator driven differentiation occurred is also suggestive of a scenario in which plants with only oil as the reward may have emerged. If we may extrapolate from the present single study case, one would expect that oil as reward was selected in the past under ecological circumstances similar to those currently favoured by selection. Consequently, it is expected to have evolved not under

circumstances where only one specialized oil-collecting bee species serviced pollination. Instead, it should have evolved where more than one oil collecting bee including generalist species serviced pollination. Supporting this view, it can be said that *Centris* oil collecting bees most probably evolved in association with Malpighiaceae and were already diverse much earlier than oil flowers emerged in Plantaginaceae (Martins et al., 2014, 2015). In addition, members of Plantaginaceae that reward exclusively with oil probably had nectar rewarding ancestors that completely lost nectaries in favour of elaiophores in subtropical and tropical regions where community diversity of oil-collecting bees was higher than in temperate latitudes (Martins et al., 2014, 2015).

Apparently, this is the first study to assess both phenotypic selection on rewards in an oil-producing plant species and response to selection in the entire distribution range of a plant species. It may be concluded that results can be explained from the perspective of a geographic mosaic of selection across the distribution range, with few hotspots immersed among a majority of coldspots. These few hotspots are located at the latitudinal extremes of the species range in consistence with the pronounced phenotypic divergence in floral rewards. An overall pattern is also supported by significant geographic analyses across latitude, abundance of the specialized pollinator and abundance of heterospecifics.

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Appendix A.

Seed production per ovary is a function of pollen receipt or pollen tubes per ovule (Aizen and Harder, 2007). The slope of this function is dependent on the quality of pollen received: with few high quality pollen grains the maximum number of seeds per ovary is attained, whereas a higher number of low quality pollen grains are needed to attain this number of seeds. Hence, seed production and pollen limitation, are particularly sensible to variation in pollen receipt (or pollen tubes per stigma) when this is of low quality. For *Monttea aphylla* pollen tubes per ovule is equivalent to pollen tubes per stigma since ovaries have one fertile ovule. For this reason we tested the relationships between the mean number of pollen tubes per stigma and fruit set focusing on pollen limited individuals, i.e. with lowest mean number of fruits per flower. Across the whole range of pollen tubes per stigma (0–300 pollen tubes/stigma) and for the 15 more strongly limited individuals from 98 individuals and five populations we tested the relationship between the number of pollen tubes per stigma and the number of fruits per flower. This test showed a significant dependence of fruit production (equiva-

lent to seed production in *M. aphylla*) from the number of pollen tubes per stigma among these more strongly limited individuals.

glm(formula = fruit/flower ~ pollen tubes/stigma, family = quasipoisson(log))

Estimate Std. Error t value Pr(>|t|)

pollen tubes/stigma 0.20914 0.04512 4.635 0.000467

Appendix B.

Univariate phenotypic selection on reward variables in each of the 16 *Monttea aphylla* populations through the pollination success variable “pollen tubes”. Linear and quadratic selection differentials (S_i and C_i , respectively) and their standard errors (SE) are included in the table. Populations are ordered by decreasing latitude. Statistical significance was obtained with the generalized linear model (GLM).

Population	Trait <i>i</i>	S_i (SE)	C_{ii} (SE)
Ampimpa	Oil vol.	0.352 (-0.189) *	0.538 (0.295) *
	Nectar vol.	0.159 (0.168)	0.142 (0.207)
Barranca Larga	Oil vol.	-0.004 (0.166)	-0.019 (0.480)
	Nectar vol.	0.113 (0.193)	0.070 (0.410)
Cuesta de Miranda	Oil vol.	-0.104 (0.156)	-0.056 (0.178)
	Nectar vol.	0.166 (0.152)	0.560 (0.409)
Tambillos	Oil vol.	-0.269 (0.275)	-0.420 (0.503)
	Nectar vol.	-0.011 (0.283)	0.922 (0.640)
Chiflón	Oil vol.	0.419 (0.340)	-1.135 (0.781)
	Nectar vol.	-0.264 (0.345)	0.451 (0.746)
Hualilán	Oil vol.	-0.035 (0.159)	-0.646 (0.363) [†]
	Nectar vol.	0.080 (0.154)	0.545 (0.344)
Estancia Maradona	Oil vol.	-0.006 (0.257)	0.017 (0.262)
	Nectar vol.	0.032 (0.279)	-0.081 (0.421)
Cadillo	Oil vol.	0.117 (0.168)	-0.107 (0.250)
	Nectar vol.	0.321 (0.153) *	0.510 (0.390)
Potrerillos	Oil vol.	0.167 (0.235)	0.070 (0.664)
	Nectar vol.	0.405 (0.309)	1.326 (0.736) [†]
Gral Alvear	Oil vol.	0.066 (0.186)	-0.171 (0.472)
	Nectar vol.	-0.116 (0.153)	-0.533 (0.288) [†]
25 de Mayo	Oil vol.	0.023 (0.156)	-0.197 (0.281)
	Nectar vol.	0.158 (0.156)	0.324 (0.169) [†]
Lihuel Calel	Oil vol.	-0.140 (0.148)	0.032 (0.295)
	Nectar vol.	-0.159 (0.130)	-0.149 (0.247)
Cutralcó	Oil vol.	-0.306 (0.162)	-0.020 (0.521)
	Nectar vol.	0.203 (0.301)	-1.118 (1.125)
Conesa	Oil vol.	-0.239 (0.317)	-0.624 (0.770)
	Nectar vol.	0.764 (0.290) *	0.935 (0.645)
Valcheta	Oil vol.	0.274 (0.190)	0.495 (0.374)
	Nectar vol.	0.313 (0.216)	0.245 (0.747)
Sierra Grande	Oil vol.	-0.121 (0.147)	-0.054 (0.180)
	Nectar vol.	-0.170 (0.153)	-0.150 (0.251)

[†] $P < 0.1$.

* $P < 0.05$.

Appendix C.

Multivariate phenotypic selection on oil and nectar volume in each of the 16 *Monttea aphylla* population through the pollination success variable “pollen tubes”. Linear, as well as correlational selection gradients (β_i and γ_{ij} , respectively) are included in the table with their standard errors (SE).

Population	Trait <i>i</i>	β_i (SE)	γ_{ij} (SE)
Ampimpa	Oil vol.	0.337 (0.222) [†]	nectar.vol.
	Nectar vol.	0.024 (0.185)	-0.54 (0.245) *
Barranca Larga	Oil vol.	-0.045 (0.181)	0.127 (0.489)
	Nectar vol.	0.132 (0.213)	
Cuesta de Miranda	Oil vol.	0.152 (0.217)	-0.243 (0.337)
	Nectar vol.	0.202 (0.157)	
Tambillos	Oil vol.	-0.278 (0.289)	0.522 (0.544)
	Nectar vol.	0.046 (0.290)	
Chiflón	Oil vol.	0.373 (0.363)	0.081 (0.586)
	Nectar vol.	-0.158 (0.359)	

Hualilán	Oil vol.	−0.0463 (0.164)	0.053 (0.197)
	Nectar vol.	0.085 (0.159)	
Estancia Maradona	Oil vol.	−0.057 (0.264)	0.715 (0.737)
	Nectar vol.	0.032 (0.287)	
Cadillo	Oil vol.	−0.025 (0.173)	0.467 (0.285)
	Nectar vol.	0.332 (0.173) †	
Potrerillos	Oil vol.	0.227 (0.231)	−0.140 (0.554)
	Nectar vol.	0.459 (0.314)	
Gral Alvear	Oil vol.	0.0038 (0.194)	−0.300 (0.453)
	Nectar vol.	−0.109 (0.161)	
25 de Mayo	Oil vol.	0.004 (0.157)	−0.168 (0.276)
	Nectar vol.	0.158 (0.162)	
Lihuel Calel	Oil vol.	−0.203 (0.148)	0.267 (0.316)
	Nectar vol.	−0.208 (0.132)	
Cutralc6	Oil vol.	−0.317 (0.163)	−0.381 (0.486)
	Nectar vol.	0.243 (0.281)	
Conesa	Oil vol.	−0.244 (0.275)	0.488 (0.667)
	Nectar vol.	0.765 (0.292) †	
Valcheta	Oil vol.	0.211 (0.197)	−0.373 (0.442)
	Nectar vol.	0.243 (0.225)	
Sierra Grande	Oil vol.	−0.042 (0.182)	−0.107 (0.514)
	Nectar vol.	−0.144 (0.193)	

†*P* < 0.1.**P* < 0.05

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