



Clinal variability of oil and nectar rewards in *Monttea aphylla* (Plantaginaceae): relationships with pollinators and climatic factors in the Monte Desert

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Received 12 June 2014; revised 29 December 2014; accepted for publication 18 March 2015

Monttea aphylla flowers simultaneously produce oil and nectar, rewards known to differentially attract ecologically and functionally distinct pollinators. We examined whether geographical differentiation occurred for rewards, and whether this could be explained by spatially heterogeneous pollinator guilds and climate. Rewards were measured across the entire species range. Geographical patterns of reward quantity and their relationships with biotic and abiotic factors were examined using uni- and multivariate analyses. Latitude significantly explained population variation in nectar and oils, although in contrasting ways. Pollinator assemblages showed a prevalence of oil-collecting *Centris* bees. *Centris vardyorum*, specialized on *M. aphylla*, showed a latitudinal pattern of visits. Oil production was not higher where plants associated with ecologically specialized bees (i.e. *C. vardyorum*), but this occurred instead where they were less frequent and co-occurred with other bees that used many floral sources, including other plant species that produce oil. The multivariate analysis showed that the prevalence of *C. vardyorum* was the factor that contributed most significantly to the combined patterns of rewards. We suggest that large-scale variation in rewards involves local optima throughout the species range, related to processes that operate in each ecoregion with their particular biotic and abiotic scenarios. © 2015 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2015, 178, 314–328.

ADDITIONAL KEYWORDS: abiotic gradient – clinal pattern – floral rewards – geographical range – oil-collecting bees – pollinator composition – specialized pollination.

INTRODUCTION

Throughout evolutionary history, in order to attract pollinators, plants have resorted to different kinds of floral reward, such as nectar, pollen, oil, resins, fragrances and food bodies (Simpson & Neff, 1981; Willmer, 2011). The kind of reward of a given plant has profound consequences on the diversity and functional composition of the pollinator assemblages (Armbruster, 2012; Martins, Aguiar & Alves-dos-

Santos, 2013). Transitions between reward systems have occurred pervasively among angiosperms, which often have substituted one reward type for another, but have rarely added a new to a pre-existing reward. Having only one kind of reward instead of several obviously limits the diversity and functional composition of the pollinator assemblages. Little is known about the ecological scenarios that have led plants to substitute one reward type for another or to reduce the number of rewards offered, and to eventually become specialized (but see Steiner, 1998; Johnson, Peter & Ågren, 2004).

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Oil secretion by flowers as a reward to pollinators has evolved at least 28 times among angiosperm families in tropical and temperate ecosystems (Vogel, 1974; Buchmann, 1987; Renner & Schaefer, 2010). This floral reward consists of fatty oils collected by a narrow functional group of solitary oil-bees which bear specialized oil-collecting structures and forage oil for larval feeding and nest building (Vogel, 1974; Simpson & Neff, 1981; Buchmann, 1987; Steiner & Whitehead, 1991; Vogel & Cocucci, 1995; Rasmussen & Olesen, 2000; Machado, 2004). Floral oil stands in contrast with the more frequently given rewards of nectar and pollen, which may be collected by a wide range of functionally disparate pollinators of several orders (Baker & Baker, 1983; Thorp, 2000; Pacini, Nepi & Vesprini, 2003). The evolutionary acquisition of oils has often involved the loss of nectaries from nectar-rewarding ancestors (Tate *et al.*, 2009; Renner & Schaefer, 2010), and thus the simultaneous offering of nectar and oil is extremely rare (Steiner, 1985; Sérsic & Cocucci, 1999; Schaefer & Renner, 2010). Consequently, plants substituting nectar with oil have become ecologically narrow in pollination relationships.

Supposing that, prior to the loss of nectar, both rewards coexisted in a certain plant, even if this occurred briefly in evolutionary time, it would be of considerable interest to know how the variation in biotic and abiotic scenarios was related to the transition in reward type. Despite being unable to obtain much knowledge about the scenarios of transitions that took place in the past, we can still study populations which, at present, are in a state of transition. Widely distributed species are made up of populations subject to geographically changing biotic and abiotic scenarios, which form instructive examples of how a species adapts locally to varying selective forces. Using such a study subject, it may then be possible to explore which pollination environment favours phenotypic specialization. For example, under the premise of a reciprocal adaptation existing between two coevolving mutualists (Armbruster, 2006; Pauw, Stofberg & Waterman, 2009; Cosacov, Cocucci & Sérsic, 2014), we would expect phenotypic specialization to be favoured where a highly specialized bee is the only or major pollinator. Although plants with dual reward systems, i.e. with nectar and oil in the same flower, are ideal study subjects in this context, we are almost completely ignorant about their ecological relationships, as they are extremely rare and have seldom been studied in detail with regard to variations in the different kinds of reward (but see Baranzelli *et al.*, 2014).

A geographical perspective in the study of plant–pollinator interactions can provide valuable insights into the dynamics of the ecological and evolutionary

processes that occur among populations (Grant & Grant, 1965; Stebbins, 1970; Herrera, Castellanos & Medrano, 2006). Related to this, several studies have revealed intraspecific floral variation associated with geographical gradients (Mascó, Noir-Meyr & Sérsic, 2004; Pérez-Barrales, Arroyo & Armbruster, 2007; Chalcoff, Ezcurra & Aizen, 2008; Nattero, Sérsic & Cocucci, 2011; Cosacov, Cocucci & Sérsic, 2012; Paiaro *et al.*, 2012a; Baranzelli *et al.*, 2014; Cosacov *et al.*, 2014), with population divergence having been recognized as the result of spatially variable pollination environments in some cases (Herrera *et al.*, 2006; Cosacov, Nattero & Cocucci, 2008; Cosacov *et al.*, 2014). In particular, floral reward phenotypes may reflect adaptations to local pollinator guilds, which can be functionally defined by their nutritional and ecological requirements (Simpson & Neff, 1983; Buchmann, 1987; Fenster *et al.*, 2004; Machado, 2004; Petanidou, 2005; Pauw, 2006). Furthermore, floral neighborhood in a community may underlie geographical variation of pollinator guilds (Sargent & Ackerly, 2008; Lázaro, Lundgren & Totland, 2009). For example, the relative abundance of oil-producing plant species, which might change in their composition along a geographical range, may affect patterns of pollinator visitation of oil-collecting bees, and indirectly drive divergence in floral rewards.

In addition to pollinator-driven differentiation, the abiotic conditions present along a geographical gradient may promote reward divergence among populations. Evidence of a congruent association between floral reward characteristics and abiotic conditions has been found for several plant–pollinator systems, even though this was exclusively related to nectar flowers (Devoto, Montaldo & Medan, 2006; Chalcoff *et al.*, 2008; Paiaro *et al.*, 2012b). Consequently, any study of geographical differentiation of the floral phenotype must account for both the biotic and abiotic factors of differentiation across populations.

Flowers of *Monttea aphylla* (Miers) Benth. & Hook.f. (Plantaginaceae) have an unusual mixed reward system, simultaneously producing oil and nectar in a single flower (Simpson, Neff & Dieringer, 1990; Sérsic & Cocucci, 1999). This dual reward system occurs in all three species of *Monttea* Gay (Sérsic & Cocucci, 1999; Baranzelli *et al.*, 2014), being sister to a clade containing genera that reward only with oil (Albach, Meudt & Oxelman, 2005). *Monttea aphylla* is visited by oil-collecting bees of the genus *Centris* (Apidae: Centridini) and non-oil-collecting bee species of several other genera (Simpson *et al.*, 1990; Sérsic & Cocucci, 1999; Tadey, 2011; Chalcoff *et al.*, 2012). Females of *Centris* use floral oil from this plant species as a resource in

addition to nectar, whereas males only visit flowers to obtain nectar (Sérsic & Cocucci, 1999; Giannini *et al.*, 2013). A frequent pollinator of *M. aphylla* is *Centris vardyorum*, a highly specialized bee, which is known from several reports to visit only this plant species in various locations (Sérsic & Cocucci, 1999; Tadey, 2011; Chacoff *et al.*, 2012). Other previously recorded pollinators include the predominantly generalist bees *C. brethesi* and *C. tricolor*, which have been reported to have several nectar and oil sources (Michelette & Camargo, 2000; Tadey, 2011; Chacoff *et al.*, 2012; A. Ferreiro *et al.*, pers. observ.).

Monttea aphylla is found in contrasting ecological conditions across its extended distribution range, which closely overlaps the Monte Phytogeographical Province (Cabrera, 1971). This ecoregion can be divided into two sectors: the subtropical and montane 'northern Monte', with summer rainfall; and the plain and temperate 'southern Monte', with weak rainfall seasonality (Prohaska, 1976; Abraham *et al.*, 2009; Labraga & Villalba, 2009).

The wide geographical distribution and the complexity of possible factors affecting this plant–pollinator system provide the opportunity to explore different scenarios in which potential biotic and abiotic factors have promoted differentiation in floral rewards. With regard to the biotic context, pertinent questions needing to be addressed are as follows. First, is the production of floral oil high in populations in which a more specialized bee (*C. vardyorum*) is the only or major pollinator? According to previous studies, one might expect that the selective pressures exerted on flowers by the specialized bee would promote the production of the specialized reward (Armbruster, 2006; Cosacov *et al.*, 2014). Second, is oil production indirectly affected by the plant community context, such that the presence of alternative oil sources may affect patterns of pollinator visitation and, indirectly, the reward amount? For example, it has been suggested through niche theory that co-occurring species will tend to use different pollinator resources and eventually become specialized in phenotype (see Pauw, 2013).

With regard to the abiotic context, the questions to be answered are as follows. First, is the variation in floral reward attributable to changes in the abiotic conditions, such as the temperature and precipitations along the geographical range of the species? Second, is oil or nectar production favoured in any of the Monte Desert ecological regions?

In this study, we explored the variation in floral oil and nectar volume in *M. aphylla* across the entire distribution range of the Monte Desert. In particular, the aims of the current study were as

follows: (1) to assess the extent of phenotypic variation in oil and nectar volume within and among populations of *M. aphylla*; (2) to assess the extent of spatial variation in pollinator assemblages, co-occurring oil-producing plant communities and climatic factors across the species range; (3) to test for associations between oil and nectar volumes across populations with differences in biotic and abiotic factors; and (4) to determine which is the most probable driver of variation (biotic vs. abiotic) in floral reward traits.

MATERIAL AND METHODS

PLANT SPECIES AND STUDY SITES

Monttea aphylla (Plantaginaceae) is an endemic species of the Monte Desert of Argentina (Rossow, 1985). The oil-secreting area, 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 (Fig. 1). The nectary is located at the base of the corolla tube, forming a thick-lobed ring between the ovary and the non-glandular corolla (Fig. 1). Previous studies at the intra-population level have reported either small quantities of nectar (0.06–0.16 μ L; Sérsic & Cocucci, 1999) or no nectar at all (Tadey, 2011). The one-seeded fruit is surrounded by the yellow and fleshy persistent calyx. *Monttea aphylla* is strictly self-incompatible and depends on pollinators to set fruit (Tadey, 2011).

Seventeen populations of *M. aphylla* were studied, covering the geographical range of the species, with sites being located approximately between 26°S and 41°S and between 64°W and 69°W, at elevations ranging from 86 to 2531 m. The sampling area covered a north–south 1671-km extension (Supporting information Appendix S1), and sampling was carried out during the flowering period from October to December 2009–2011. For each population, geographical coordinates were obtained *in situ* using the Global Positioning System (i.e. latitude, longitude and elevation).

FLORAL REWARDS

For each population, 20 flowering individuals of *M. aphylla*, at least 5 m from each other, were selected to assess floral reward production. From each plant, three recently opened unvisited flowers were collected at random at 8 a.m. in the morning to measure the nectar and oil volumes. We selected freshly opened flowers with dehiscent anthers that showed the complete pollen load, which indicates

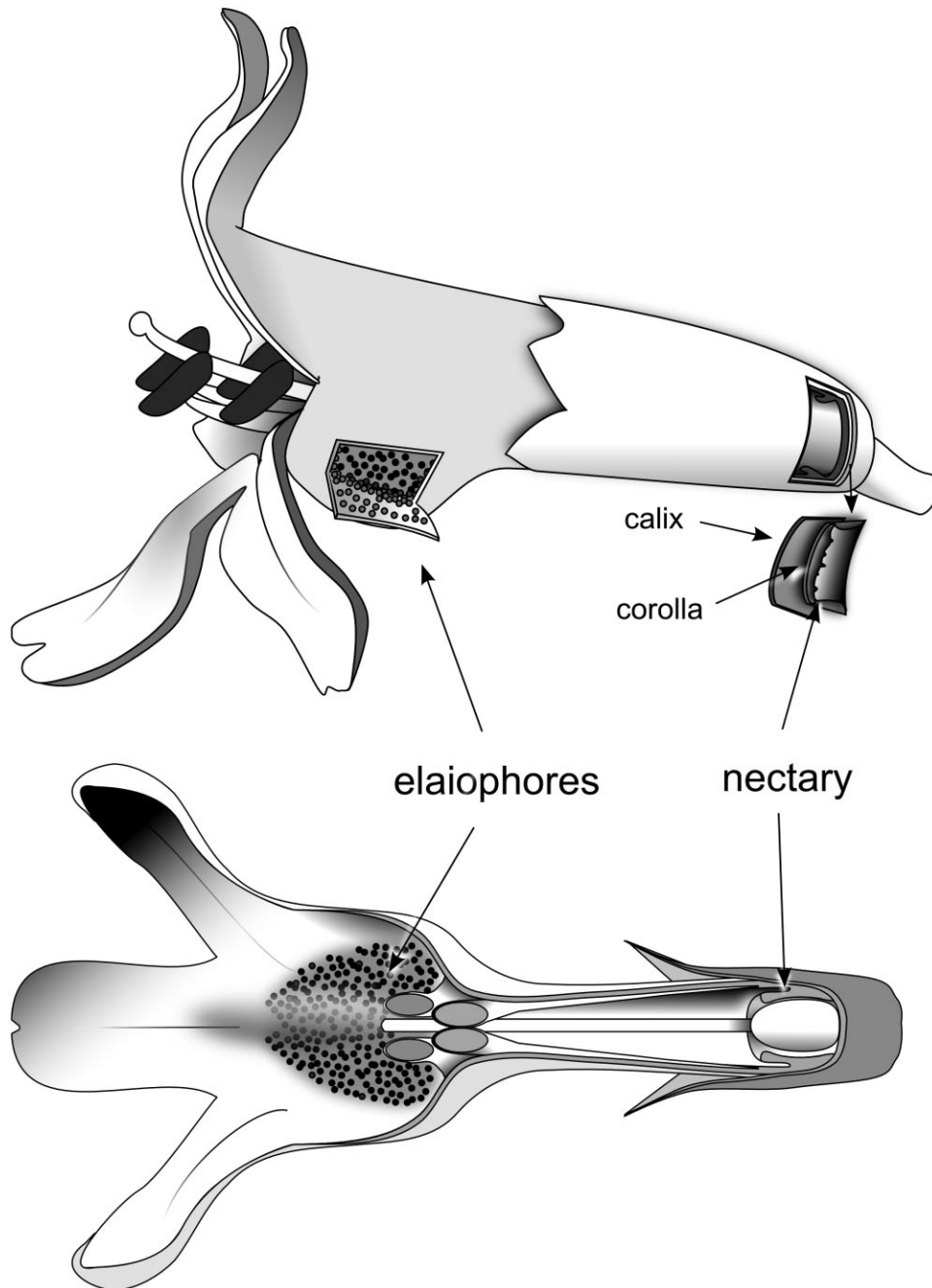


Figure 1. Schematic diagram of a *Monttea aphylla* flower showing the elaiophore and nectary location.

that flowers had not been visited. In total, 1020 flowers and 340 individuals from 17 populations were sampled.

For each flower, two variables were measured. Nectar was extracted in 1- μ L capillary tubes and the nectar volume was recorded (Drummond microcaps). The oil volume was estimated from oil collected with

a cigarette rolling paper, which was gently pressed onto the oil-secreting portion of the corolla until saturated. After allowing the paper to dry for at least 24 h, oil stains on the paper were photographed using a scale, and their volume was estimated with a calibration curve (Supporting information Appendix S2), obtained with castor oil on the same type of

cigarette paper, which was chosen for its chemical and physical similarities to floral oil (Vogel, 1974; Seipold, 2004).

POLLINATOR ASSEMBLAGES AND CO-FLOWERING OIL-PRODUCING PLANTS

To determine the pollinator assemblage composition, direct observations of pollinator visits were made for 15 min on 12 individuals at each of the 16 study sites (180 min per site) between 8:30 h and 16:30 h, thus including the time of greatest bee activity. Analyses on visitation frequencies are restricted to 16 populations because visitation frequencies could not be obtained in the Villavencio site. To exclude any possible temporal change in the pollinator assemblage composition, observations were made during two consecutive years in three populations, which revealed that the pollinator composition of the main visitors did not vary between years (Supporting information Appendix S3). At least one voucher of each flower visitor species was collected for later identification by comparison with local collections and available literature (Roig-Alsina, 2000). Floral visitation frequency was calculated as the number of flowers visited/number of flowers observed/observation time in minutes. In addition, we distinguished between visits made by males and females of *Centris* spp., because they forage flowers searching for different rewards: males visited *M. aphylla* flowers exclusively for nectar, whereas females visited flowers searching for both rewards. We also discriminated between the visits of the females of different *Centris* spp., taking into account their ecological differences in foraging behaviour recorded in previous studies (Michelette & Camargo, 2000; Tadey, 2011; Chacoff *et al.*, 2012). The following six pollinator variables were considered: (1) floral visitation frequency of *Centris* females (VF♀*Centris* spp.); (2) floral visitation frequency of *Centris* males (VF♂*Centris* spp.); (3) floral visitation frequency of other visitor species (VF other visitors); (4) VF♀*C. vardyorum*; (5) VF♀*C. tricolor*; and (6) VF♀*C. brethesi*.

In addition, we estimated the relative abundance of co-flowering oil-producing plant species in the community to account for other oil sources sharing oil-collecting bees with *M. aphylla*. To analyse the co-flowering neighbourhood and particularly to determine the relative abundance of other oil-producing plant species, two transects of 2 × 50 m were used at each site. In each transect, the abundance of each blooming plant species was determined. Vouchers are deposited in the Botanical Museum of Córdoba, Argentina (CORD).

CLIMATIC DATA

For the geo-referenced locations of each population, climatic data were obtained from the WorldClim database (Hijmans *et al.*, 2005). We selected the following six measures, which were considered to be biologically meaningful given their possible influence on the variation in floral rewards: maximum and minimum temperature of the flowering season (Max.TFS and Min.TFS, respectively) and precipitation in the flowering season (PFS), obtained from data for the flowering months, i.e. October to December (Tadey, 2011), annual temperature (AT), annual precipitation (AP) and precipitation in the cold season (PCS), which represent the main climatic differences between ecoregions in the Monte (Prohaska, 1976; Labraga & Villalba, 2009; Appendix S1).

STATISTICAL ANALYSES

Structure of geographical variation in floral rewards

To partition the total variance of reward traits into its hierarchical components, a nested analysis of variance (ANOVA) with random effects was performed for each measured trait. Each reward trait was partitioned according to its differences among populations, individuals within populations and flowers within individuals (the last level was used as the error term). We tested for significant differences at each level using the restricted maximum likelihood (REML) estimation (Zuur *et al.*, 2009). To explore whether floral rewards varied in a latitudinal cline across the species geographical range, simple regression models were used between population means of each floral reward variable and latitude across populations.

Geographical variation in biotic and abiotic factors

Univariate regression models across populations were applied to biotic and abiotic variables (i.e. pollinator assemblages, co-flowering oil-producing plant communities and climatic factors) with latitude as the independent variable.

Relationships between geographical variation in floral rewards and biotic factors

First, Spearman correlation analyses were performed between population means of each floral reward variable and biotic variables (i.e. pollinator visitation frequencies and relative abundance of co-flowering oil-producing plants). Second, to determine whether the visitation frequency of females of *Centris* spp. and the relative abundance of co-flowering oil-producing plants were affecting reward quantity, a multiple linear regression was performed for each response variable separately (oil and nectar volume), including these two predictor variables and the interaction

between them. The statistical significance was estimated using generalized linear models with a gamma error distribution (Zuur *et al.*, 2009). Third, to test whether or not the floral reward variables differed with respect to the prevalence of the single highly specialized bee (*C. vardyorum*), generalized linear models with a gamma error distribution were used considering the following classificatory variable, prevalence or not of *C. vardyorum*, with *C. vardyorum* being considered to prevail when its relative visitation frequency was greater than 50%.

Relationships between geographical variation in floral rewards and abiotic factors

First, we performed Spearman correlation analyses between the population means of each floral reward variable and climatic variables. Second, generalized linear models with a gamma error distribution were used to test whether floral reward variables differed in relation to their location for each ecoregion, with a classificatory variable being defined by the localization of the populations at latitudes <37°S (northern Monte) or >37°S (southern Monte).

Multivariate patterns and possible factors of reward variation

To analyse the joint association of floral reward variables with biotic and abiotic factors, a multivariate analysis of redundancy (RDA) was performed. RDA is a multivariate ordination method in which the axes are constructed by a linear combination of predictor variables. For this analysis, a matrix of population × reward variables was analysed in relation to a corresponding matrix of explanatory biotic and abiotic variables. We included those biologically relevant variables that were significantly associated with latitude or with rewards in univariate analyses (see below). The significance of the variation explained by

each environmental factor was analysed by the automatic selection of variables using a Monte Carlo test with 999 permutations. In this procedure, the variable that best fits the data is selected first, and then the next best fitting variable is added to the model (Oksanen *et al.*, 2013).

Statistical analyses were performed using R v.3.0.3 statistical software (R Development Core Team, 2014). For the nested ANOVA, we used the *lme4* package (Bates & Maechler, 2010), whereas, for RDA, we used the *vegan* package (Oksanen *et al.*, 2013).

RESULTS

GEOGRAPHICAL VARIATION IN FLORAL REWARDS

Nested ANOVA performed on each floral reward showed that the greatest proportion of variance (42–44%) was explained by variation among populations, whereas a relatively small, but still significant, proportion was explained by variation among individuals within populations (Table 1). The mean nectar volume per population ranged from 0.05 to 1.25 µL, with oil volume ranging from 0.16 to 1.21 µL. Simple regression models revealed significant clinal patterns of variation in the floral rewards along the latitudinal gradient (Fig. 2A). Nectar volume increased significantly with latitude, whereas oil volume showed the opposite pattern (Fig. 2B, C).

GEOGRAPHICAL VARIATION IN POLLINATOR ASSEMBLAGES, CO-FLOWERING OIL-PRODUCING PLANTS AND CLIMATIC FACTORS

Pollinator assemblages revealed a 74–100% prevalence of *Centris* bees in all populations. In five of the 16 populations (31%), the pollinator assemblages were composed exclusively of bees of this genus, either

Table 1. Results of the nested analysis of variance (ANOVA) based on reward volume data. Degrees of freedom (d.f.), variance components and percentage of total variance are given for each hierarchical level: among populations, among individuals within populations and within individuals (error term)

Source of variation	d.f.	Variance components		% of total variance	
		Oil volume	Nectar volume	Oil volume	Nectar volume
Among populations	16	0.075	0.123	44.56*	42.90*
Within populations	323	0.044	0.063	26.01*	21.83*
Within individuals	680	0.049	0.101	29.43	35.27
Total		0.168	0.286		

* $P < 0.001$.

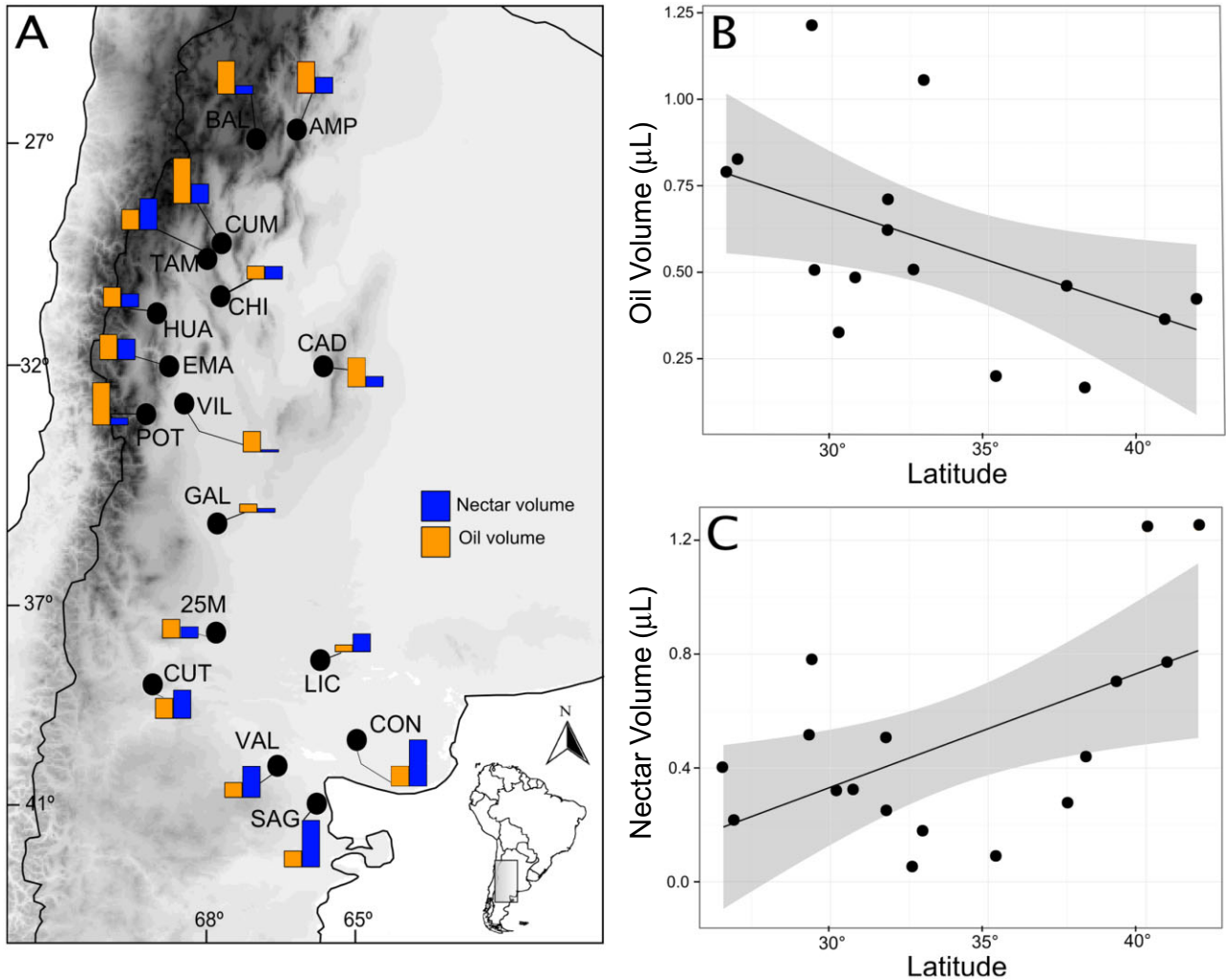


Figure 2. A, Geographical variations in nectar and oil volume among 17 populations of *Monttea aphylla* throughout their distributional range in the Monte Desert (Argentina). Bars show the mean values for the oil and nectar volumes (expressed in microlitres). B, C, Regression analyses between oil ($R^2 = 0.23$, $P = 0.03$) and nectar ($R^2 = 0.27$, $P = 0.02$) volumes and the geographical latitude throughout the distributional range of *M. aphylla*. The grey sections represent 95% confidence intervals.

females and males or only females, whereas, in the remaining 11 populations (69%), other nectar-collecting species were also recorded (Fig. 3A; see visitation frequencies of each pollinator per population in Appendix S3). Oil-collecting bees showed a population variation in the abundance of the species *C. vardyorum*, *C. brethesi* and *C. tricolor* (Fig. 3A), but only VF♀ *C. vardyorum* increased with a marginal significance with latitude (Fig. 3B).

Monttea aphylla was the only or dominant oil source in all populations (Fig. 3C). Two co-flowering oil-producing species, *Tricomaria usillo* Hook. & Arn. and

Monttea schickendantzii Griseb., were present in three and two northern populations of *M. aphylla*, respectively (Fig. 3C). Furthermore, in all studied populations, other nectar-producing plants were present as additional nectar sources to pollinators (Supporting information Appendix S4). Accordingly, the relative abundance of co-flowering oil-producing plants decreased with latitude ($R^2 = 0.18$, $P = 0.06$). With regard to climatic factors, only PCS increased significantly with latitude (Fig. 3D), showing that the southern populations have the availability of water throughout the year.

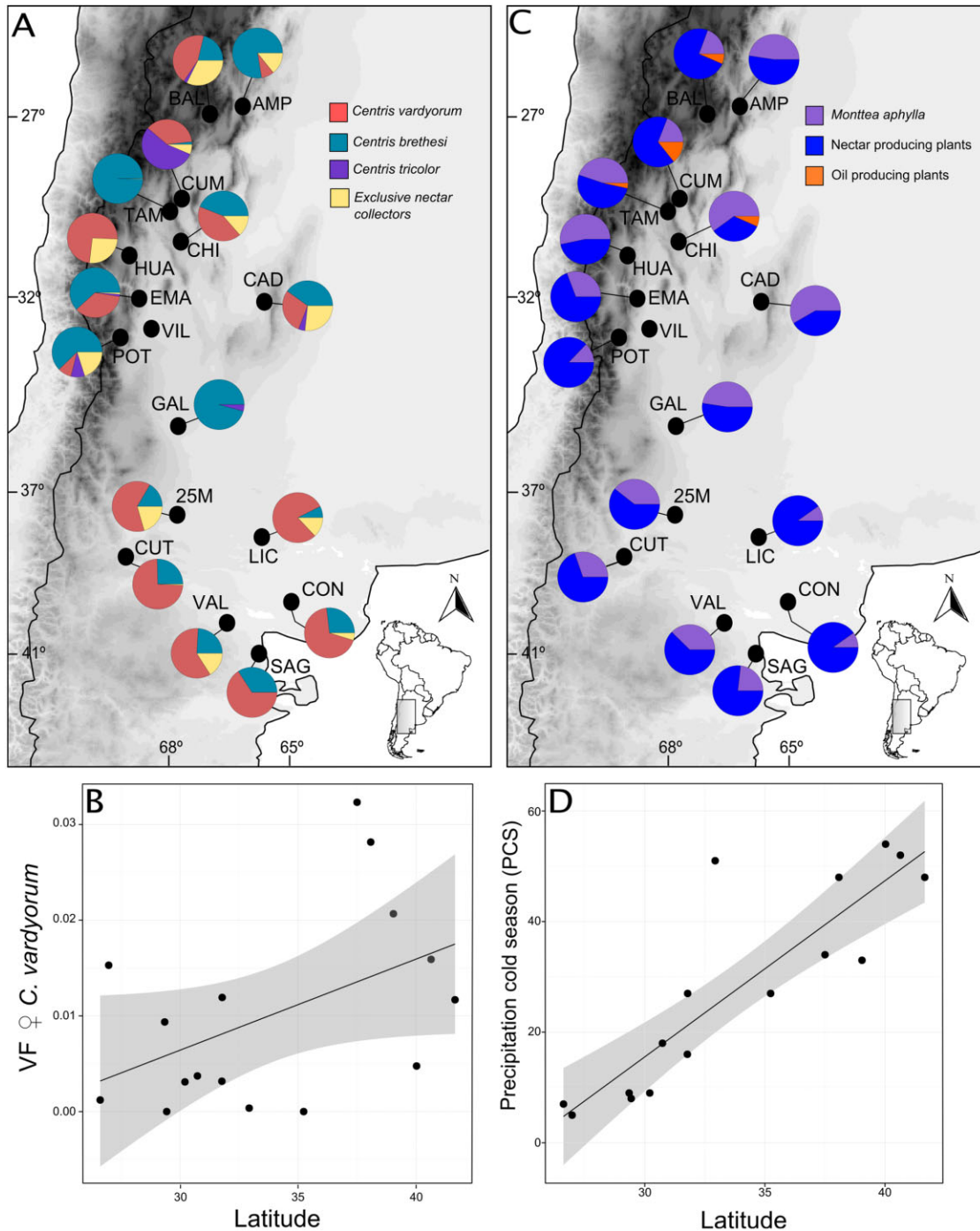


Figure 3. Geographical variations in biotic and abiotic factors throughout the distributional range of *Monttea aphylla*. A, Relative abundance of floral visits corresponding to females (♀) of the different species of *Centris* (oil- and nectar-collecting visitors) and exclusive nectar foragers (males of *Centris* spp. and other nectar-collecting species). The relative abundance is quantified as the proportion of floral visits/total visits. Full location names are provided in Appendix S1. Visitation frequencies corresponding to each pollinator are provided in Appendix S2. B, Regression analyses between VF♀*C. vardyorum* and the geographical latitude throughout the distributional range of *M. aphylla* ($R^2 = 0.17$, $P = 0.06$). C, Relative abundance of *M. aphylla* and co-flowering oil- and nectar-producing plants. Relative abundances were quantified as the proportion of plants in flower in relation to the total plants flowering in the community. D, Regression analyses between precipitation in the cold season (PCS) and the geographical latitude ($R^2 = 0.75$, $P < 0.001$). The grey sections represent 95% confidence intervals.

Table 2. Results of the multiple regression analysis performed for each reward type, including the visitation frequency of females of *Centris* spp., the relative abundance of co-flowering oil-producing plants (R.a.oil-plants) and the interactions between both predictor biotic variables. Statistical significance was obtained with the generalized linear model (GLM)

Biotic variables	Oil volume	Nectar volume
	B_i (SE)	B_i (SE)
VF♀ <i>C. vardyorum</i>	-10.58 (5.90)*	0.94 (10.38)
R.a.oil-plants	-2.95 (1.74)	0.45 (3.06)
VF♀ <i>C. vardyorum</i> × R.a.oil-plants	596.38 (200.81)†	-141.55 (353.24)
VF♀ <i>C. tricolor</i>	174.06 (140.57)	-316.5496 (205.66)
R.a.oil-plants	-0.29 (1.37)	-1.53 (2.01)
VF♀ <i>C. tricolor</i> × R.a.oil-plants	-548.86 (665.96)	1553.52 (974.35)
VF♀ <i>C. brethesi</i>	13.66 (17.51)	-12.79 (23.08)
R.a.oil-plants	2.47 (1.43)	-1.45 (1.88)
VF♀ <i>C. brethesi</i> × R.a.oil-plants	-235.44 (274.21)	241.96 (361.41)

SE = standard error.

* $P < 0.1$; † $P < 0.05$.

FLORAL REWARD RELATIONSHIPS WITH BIOTIC AND ABIOTIC FACTORS

The correlation analysis between floral reward and biotic variables showed that reward variables exhibited significant correlations with VF♀*C. tricolor*, being positive for oil volume, but negative for nectar volume ($r = 0.561$, $P = 0.02$ and $r = -0.5188$, $P = 0.04$, respectively; Supporting information Appendix S5). Furthermore, the multiple regression analyses that included the visitation frequency of females of *Centris* spp. and the relative abundance of co-flowering oil-producing plants as predictor variables showed that VF♀*C. vardyorum* was the main predictor of oil volume (Table 2). In the same model, this association was stronger in combination with the relative abundance of co-flowering oil-producing plants (Table 2). This means that oil volume increased when *C. vardyorum* was less frequent and co-occurred with many oil-producing plants. In the same way, the comparison of floral reward variables between populations with the prevalence (or not) of *C. vardyorum* showed significant differences for both reward variables, with high nectar and low oil volumes in populations in which this pollinator prevailed (Fig. 4A).

In relation to abiotic factors, the correlation analyses performed between floral rewards and climatic variables revealed only a marginally negative correlation between oil volume and temperature ($r = -0.48$; $P = 0.06$ and $r = -0.45$; $P = 0.07$, for Max.TFS and AT, respectively; Appendix S5). Moreo-

ver, the comparison of floral reward variables between populations according to ecoregions showed significant differences for nectar volume and marginally significant differences for oil volume, with high nectar and low oil volumes found in populations of the southern Monte (Fig. 4B).

MULTIVARIATE PATTERNS AND POSSIBLE FACTORS CONTRIBUTING TO REWARD VARIATIONS

For the multivariate analysis, we included those predictor variables that were significantly associated with latitude or with rewards in univariate analyses. We included the following four explanatory factors: two biotic variables (1, the prevalence or not of *C. vardyorum*; and 2, the relative abundance of oil-producing plants) and two climatic variables (3, Max.TFS; 4, PCS). VF♀*C. tricolor* was excluded given that the correlation with rewards is presumably not causal, because it is only supported by the absence of this bee in the south (see Fig. 3A). TA was also excluded because it was highly correlated with Max.TFS. The RDA results performed to analyse the associations of floral reward variables with these biotic and abiotic factors showed that the prevalence of *C. vardyorum* ($F = 4.8325$; $P = 0.02$) was the factor that most significantly contributed to the model (Fig. 5). The vector representing the direction of maximum change of this factor was the most strongly correlated to the first RDA axis.

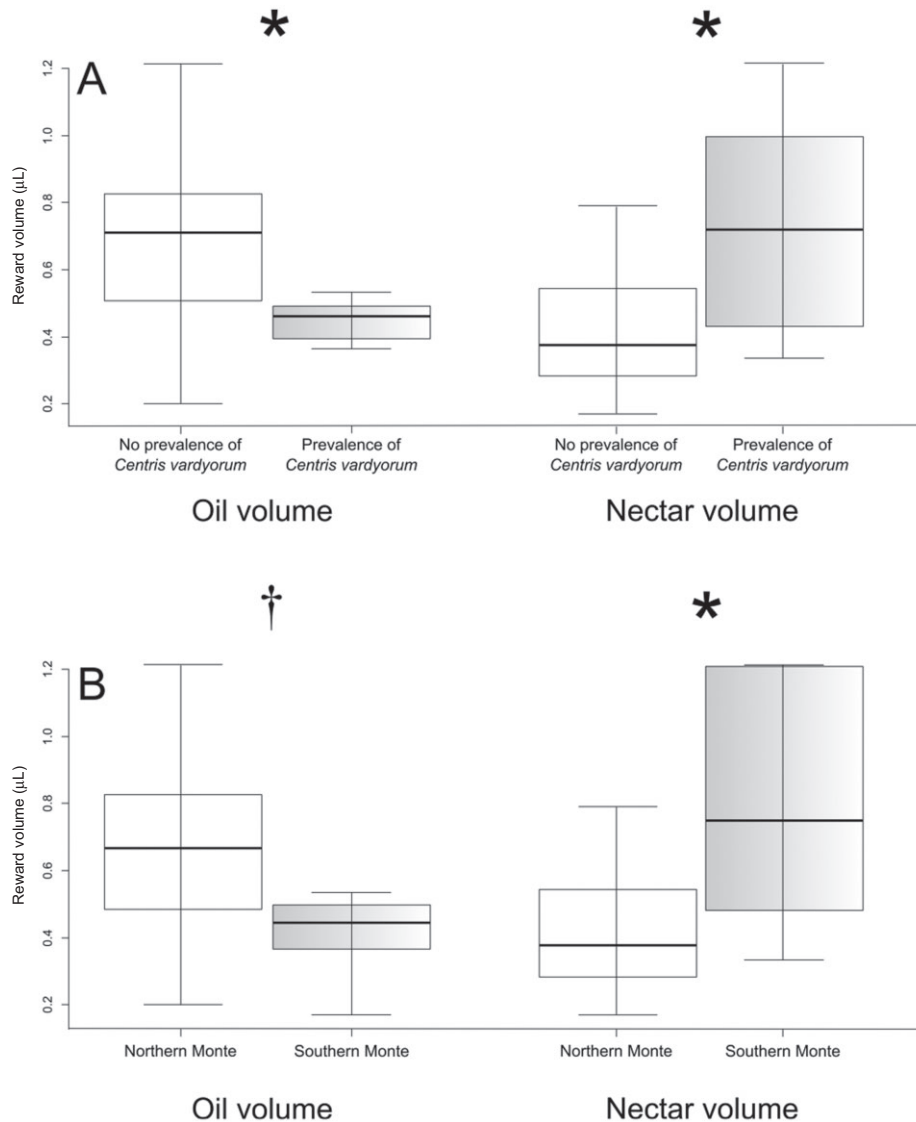


Figure 4. Box plot comparing reward variables according to the following classificatory variables. A, Prevalence or not of *Centris vardyorum* (Dev = 4.01; d.f. = 15; $P = 0.03$ and Dev = 7.05; d.f. = 15; $P = 0.04$, for oil and nectar volumes, respectively). B, Ecoregions (Dev = 4.0116; d.f. = 15; $P = 0.06$ and Dev = 7.0555; d.f. = 15; $P = 0.02$, for oil and nectar volumes, respectively). Thick full line within the box indicates the median value. Horizontal lines in the boxes represent 25%, 50% (median) and 75% values; error bars are also included. d.f., degree of freedom.

DISCUSSION

GEOGRAPHICAL VARIATION IN FLORAL REWARDS, POLLINATOR ASSEMBLAGES AND COMMUNITY CONTEXT

The present study showed a strong clinal pattern in the amount of nectar and oil rewards across the natural distribution range of *M. aphylla*. Although intraspecific variation in relation to geographical gradients has been reported previously for nectar rewards (e.g. Devoto *et al.*, 2006; Paiaro *et al.*, 2012b), this has been reported rarely for oil (Cosacov *et al.*, 2012), with little being known about the simultaneous variation of

oil and nectar on a geographical scale (Baranzelli *et al.*, 2014). Our results showed contrasting tendencies for nectar and oil volumes across the geographical range, with nectar increasing, but oil decreasing with latitude.

Population differentiation in floral traits is often attributed to variations in pollinator mosaics (Grant & Grant, 1965; Stebbins, 1970; Johnson & Steiner, 1997; Anderson & Johnson, 2008; Pauw *et al.*, 2009). Thus, a local floral adaptation in response to geographically divergent pollinators would be expected when functional groups of pollinators vary among

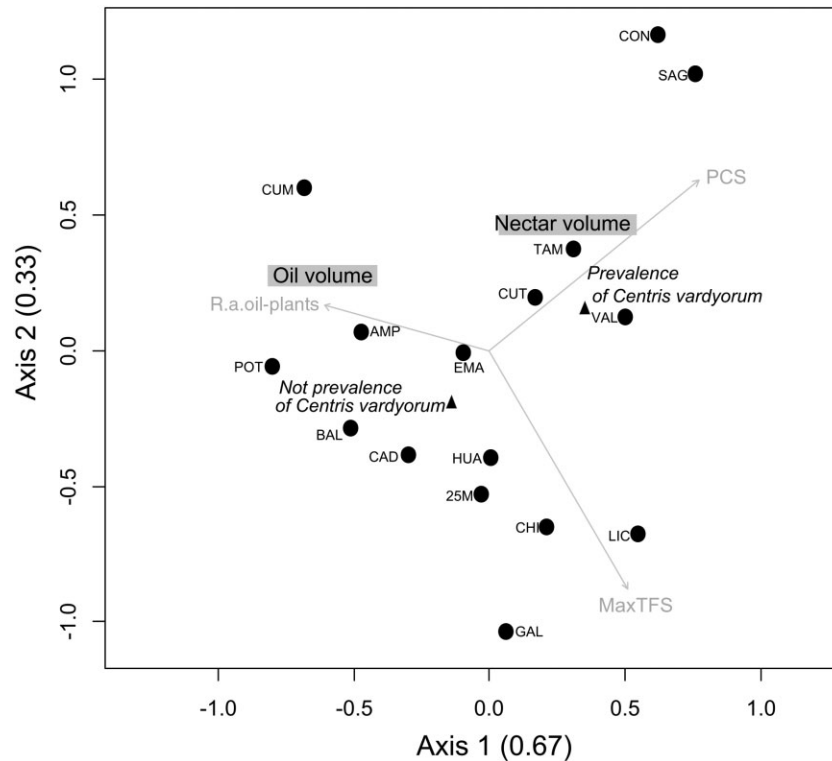


Figure 5. Biplot of the first two axes of the multivariate analysis of redundancy (RDA) ordinations of 16 populations of *Monttea aphylla* (black circles); significant ($P < 0.05$) explanatory variables included in the model (black triangles) that could be determinant in floral reward variation are also shown. Explanatory variables are detailed in the Material and methods section. The eigenvalue associated with each axis is provided in parentheses. Full names for population acronyms are given in Appendix S1. Max.TFS, maximum temperature of the flowering season; PCS, precipitation in the cold season; R.a.oil-plants, relative abundance of co-flowering oil-producing plant species.

populations (Herrera *et al.*, 2006; Anderson *et al.*, 2014; Cosacov *et al.*, 2014). In the present study, although oil-collecting bees prevailed in all studied populations, there was geographical variation in the abundance of the different oil-collecting species visiting *M. aphylla*, and, in particular, a clinal pattern was revealed for the specialized *C. vardyorum*, whose visits increased with latitude. Although our estimation of pollinator abundance is based on visitation frequencies to *M. aphylla* alone, it represents an estimate of the community abundance of oil-collecting bee species in most populations, because it is the only oil source at most sites. However, this estimate should be interpreted with caution for nectar-feeding visitors as their visitation frequencies may be strongly biased by the abundance of other nectar sources, which varied strongly across the distributional range.

The association detected between pollinator assemblages and floral rewards was unexpected, considering the possible co-evolutionary pattern in this highly specialized plant–pollinator system (Armbruster, 2012; Cosacov *et al.*, 2014). When the *M. aphylla* spe-

cialist *C. vardyorum* prevailed, flowers tended to have high nectar and low oil volumes. The opposite tendency, i.e. higher oil volume, where the generalist *C. tricolor* showed a high visitation frequency, was also found, but this association is presumably not causal, because it is only supported by the absence of this bee in the south. Furthermore, the negative association between visitation frequency of the specialized bee (*C. vardyorum*) and oil quantity was contingent with the co-flowering community contexts, such that oil seemed to increase across populations in which *M. aphylla* was not the only oil source.

These results pose the question. Why is nectar favoured in populations in which the most specialized oil-collecting bee prevails, but oil is favoured in populations with pollinators that use many floral sources? Although the complexity of the system may not allow us to answer this question, it may be suggested that phenotypic specialization (oils) might be favoured in scenarios in which plants compete for the pollinator services of generalist bees. Reciprocally, relatively high nectar volumes may be locally favoured by the prevalence of the highly specialized bee (i.e. *C. vardyorum*),

where the pollination service is guaranteed by this single pollinator. Nevertheless, several other queries still remain in order to clarify the observed patterns, such as the cost–benefit balance in the production of each reward and competition for the rewards among bee functional groups, among others. For example, from a historical perspective, palaeoclimatic evidence suggests that the southern limit of the Monte Desert shifted to the north during Quaternary glacial periods (Markgraf, 1989; Mancini *et al.*, 2005; Labraga & Villalba, 2009), reducing the area of the southern Monte. Consequently, *M. aphylla* and its pollinators more recently colonized the southernmost ranges. This would mean that the reduction in oil reward and the increase in nectar reward occurred more recently in the evolutionary history of *M. aphylla* (M. C. Baranzelli *et al.*, unpubl. data). It remains to be answered whether recolonization occurred in association with a specialized pollination assemblage or whether *M. aphylla* exploited nectar feeders before *Centris* recolonized southern areas.

FLORAL REWARD RELATIONSHIPS WITH ABIOTIC FACTORS

Evidence of congruent associations between floral rewards and abiotic conditions along geographical gradients has been reported for several nectar-offering plants (Devoto *et al.*, 2006; Paiaro *et al.*, 2012b). However, in the present study, the correlation between reward amount and the considered climatic variables was not strong, with only a marginally significant correlation between oil volume and temperature, which was lost when considering the concerted contributions of all variables in the RDA. However, strong differences existed between ecoregions in both floral rewards, with populations in the southern Monte having higher nectar volumes, but lower oil volumes, than in the north. In relation to these ecoregions, we found that PCS showed a strong latitudinal pattern across the geographical range. Although the availability of water throughout the year in the southern Monte might explain the differences in floral reward quantities (see Pacini *et al.*, 2003; Devoto *et al.*, 2006), neither PCS nor any other precipitation variable explained the geographical pattern found for the rewarding system.

COMBINED CONTRIBUTION OF POLLINATOR ASSEMBLAGES AND CLIMATE

The multivariate analysis showed that the prevalence of *C. vardyorum* contributed more strongly than the co-flowering oil-producing plant community and climatic factors to the combined pattern of variation for both rewards. This suggests that the effect of the

prevalence of the specialized bee in moulding the reward balance supersedes that of other biotic and abiotic variables included in the model.

Taken as an instructive example of the evolutionary transition between nectar- and oil-rewarding species, the present study suggests that the loss of nectar in favour of oil as the reward is more likely to occur in subtropical than in temperate scenarios (i.e. northern Monte), because of the high abundance of generalized bee pollinators, with highly specialized oil-collecting bees being less abundant. This is also consistent with previous studies which have shown the predominant distribution of nectarless oil-secreting close relatives of *Monttea* in subtropical and tropical zones (Barringer, 1981, 1983, 1985; Albach *et al.*, 2005).

In summary, we have shown that oil and nectar rewards in *M. aphylla* exhibit a strong latitudinal variation. In addition, the present results suggest that oil production is not promoted where plants are associated with ecologically highly specialized bees (such as *C. vardyorum*), but, instead, is promoted where they are less frequent and co-occur with other bees that use many floral sources, including other plant species that produce oil. We propose two possible mechanisms explaining the promotion of oil as a reward. On the one hand, the low visitation frequencies of the specialized bee could set plants in intraspecific competition through pollen limitation, favouring plants that produce more oil as a reward. However, this explanation would only work if the specialized bees were better pollinators than other oil-collecting bees, because overall visitation frequencies of oil-collecting bees did not explain the pattern of variation in floral oil. On the other hand, competition for the benefits provided by generalist oil-collecting bees among plant species in communities with several oil sources could be the mechanism promoting phenotypic specialization (Sargent & Ackerly, 2008; Pauw, 2013). Our findings also indicate that the large-scale geographical variability in reward quantity probably involves different local optima throughout the species range, related to processes that operate in biotic and abiotic contexts. Future studies that focus on the relationship between fitness and the proportion of rewards in variable geographical scenarios of pollinator assemblages should be able to reveal whether pollinator-mediated phenotypic selection could eventually explain the patterns found here (see Herrera *et al.*, 2006).

ACKNOWLEDGEMENTS

We thank the Doctorate in Biology and the Secretary of Science and Technology (SECyT), both at the University of Córdoba, Argentina, where G.F. is a PhD student and fellowship holder, respectively. The

authors wish to acknowledge the assistance of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and the Universidad Nacional de Córdoba, both of which support the facilities used in this investigation, and APN Argentina for granting permits to obtain samples from national parks and reserves. We thank Dr Paul Hobson, native English speaker, for revision of the manuscript. We also thank Dr Santiago Benitez-Vieyra for his valuable input into the statistical analysis, Dr Andrea Cosacov, Dr Ana A. Calviño and Dr Valeria Paiaro for their useful comments on previous versions of the manuscript, and Nicolás de Vita for field assistance. This work was supported by the National Research Council of Argentina (PIP 11220080101264), the National Fund for Science and Technology (FONCyT, PICT 01-10952 and 01-33755) and Secretary of Science and Technology of the University of Córdoba (SECyT-UNC, Res. 214/10) to A.N.S and A.A.C.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Appendix S1. Location and climatic conditions of the *Monttea aphylla* populations studied. Max.TFS, maximum temperature of the flowering season; Min.TFS, minimum temperature of the flowering season; AT, annual temperature; PFS, precipitation in the flowering season; PCS, precipitation in the cold season; AP, annual precipitation. Data obtained from the WorldClim Global Climate GIS. Dataset available online at <http://www.WorldClim.org>. (*) Pollination assemblage was not studied in this population because visitation frequencies could not be obtained in the Villavicencio site.

Appendix S2. Calibration curve obtained to estimate the oil volume from the area of the oil stain on cigarette paper. The formula used was as follows: $\text{volume} = (10^{-0.37}) \times (10^{1.39})^{\text{area}}$.

Appendix S3. Floral visitation frequencies for the different pollinators recorded in each of the 16 *Monttea aphylla* populations studied. Populations are ordered by decreasing latitude. (*) Floral visitors observed during the second flowering season. Floral visitation frequency was calculated as the number of flowers visited/number of flowers observed/observation time in minutes.

Appendix S4. Co-flowering plant species present in each of the 16 populations of *Monttea aphylla* studied. The relative abundance of co-flowering oil plants (R.a.oil-plants) and the relative abundance of *M. aphylla* (R.a.*M. aphylla*) are shown. Populations are ordered by decreasing latitude.

Appendix S5. Spearman correlation indices between assemblages of pollinators, plant communities and climatic variables with floral reward traits. VF, visitation frequency; R.a.oil-plants, relative abundance of oil-producing plants; Max.TFS, maximum temperature of the flowering season; Min.TFS, minimum temperature of the flowering season; AT, annual temperature; PFS, precipitation in the flowering season; PCS, precipitation in the cold season, AP, annual precipitation.