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Pollination unpredictability and ovule number in a South-Andean Proteaceae along a rainfall gradient

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Abstract. Pollen limitation occurs frequently in plant populations and, as result, many ovules are wasted. One possible adaptive explanation posits that ovule overproduction represents a 'bet-hedging' strategy against pollination inefficiency. This hypothesis is supported by comparative evidence showing that unpredictability in pollen receipt is positively associated with an increasing number of ovules per flower across species. Yet, this proposition has not been tested at the intraspecific level, where natural selection operates. Here, we evaluated the relationship between pollination unpredictability, considering both pollination quantity and quality, and number of ovules per flower, across 16 populations of the south-Andean generalist treelet *Embothrium coccineum* J.R.Forster and G.Forst from north-western Patagonia, which occurs along a west–east gradient of decreasing rainfall. Despite sizable variation in mean number of ovules per flower, we found no increase in ovule production with increasing pollination unpredictability across populations. Instead, we found that mean number of ovules per flower decreased with decreasing rainfall. Therefore, in this species, there was no support for the proposal that ovule overproduction represents a bet-hedging strategy against unpredictable pollen receipt. Rather, the number of ovules per flower seems to be conditioned primarily by resource availability.

Additional keywords: among-population variation, ovule number per flower, resource availability.

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Introduction

According to theory, seed set should be equally limited by both pollen and resource availability (Haig and Westoby 1988). At equilibrium, pollen addition is not expected to increase plant fitness because resources should be unavailable for the development of any extra fertilised ovule (Ashman *et al*. 2004); however, decreasing stigmatic pollen deposition below observed levels should lead to pollination limitation. One corollary of this hypothesis is that under natural selection, resources allocated to sexual reproduction should be finely tuned with the pollination environment experienced by each plant population (but see Burd 2008; Schreiber *et al*. 2015). However, pollination limitation is widely observed in nature and, as a consequence, many ovules are wasted (Burd 1994; Ashman *et al*. 2004).

Pollination is a spatially and temporally unpredictable process (Herrera 1988), despite the many floral mechanisms that can foster pollination efficiency (Stebbins 1970). This efficiency can be measured not only in terms of pollination success (i.e. mean number of pollen grains deposited on the stigma or pollen tubes growing in the style), but also by pollination predictability (i.e. the inverse of the variability in the number of pollen grains or pollen tubes). Although the number of ovules packaged within an ovary should relate to expected mean pollination levels, it has been proposed that ovule overproduction could represent a bet-hedging strategy against pollination unpredictability (Cruden 1977; Burd 1995; Cruden 2000; Harder and Routley 2006; Schreiber *et al*. 2015). More specifically, this bet-hedging hypothesis posits that in a highly unpredictable pollination environment, plants should increase their fitness by taking maximum advantage of the few stigmas that receive large amounts of compatible pollen. Thus, selection for increasing number of ovules per flower should become stronger in populations experiencing less predictable pollination. In fact, Burd *et al*. (2009) provided comparative evidence supporting the hypothesis that pollination unpredictability is positively associated with an increasing number of ovules per flower across species. Nevertheless, this hypothesis still awaits validation at the microevolutionary scale.

If pollination unpredictability were a strong selection force determining ovule number, we might predict larger allocation of resources, when available (Burd 1995), to ovule production in flowers of populations experiencing more stochastic pollination (but see Schreiber *et al*. 2015). However, at this level, there are several factors that could obscure or neglect the role of this selective factor. Particularly, different environmental factors, e.g. water stress, might constrain both the availability of efficient pollinators, and the size and number of different plant structures, thus limiting the maximum number of ovules that can be packaged within an ovary.

The south-Andean Proteaceae *Embothrium coccineum* occurs along a strong rainfall gradient that determines compositional changes in its pollinator assemblage, from pollination-efficient, hummingbird-dominated at the rainy-west end, to pollination-inefficient, bee-dominated at the dry-east end of the distribution (Chalcoff *et al*. 2012). In this self-incompatible species, lower pollination efficiency is associated with a large increase in pollen limitation leading in fact to much reduced and sporadic seed production (Chalcoff *et al*. 2012). Therefore, according to the hypothesis of Burd *et al*. (2009), individuals at eastern populations of *E. coccineum* should be under strong selection for producing ovaries with a high number of ovules. However, in contrast, individuals at eastern populations of *E. coccineum* could be under the selective influence or respond plastically to other opposing non-pollinator agents such as drought that would limit the number of ovules per flower (Strauss and Whittall 2006, and references therein). Thus, co-varying environmental factors eventually could override any role of pollination-mediated selection on ovule number. Evidence of resource limitation of ovule production would not deny a role for pollination-related bet-hedging, although, at least in the study system, it will diminish its importance.

Here, we assessed the relationship between pollination unpredictability and ovule number per flower across 16 populations of *E. coccineum*. We had the following three main objectives: (1) to evaluate whether populations diverge in the number of ovules per ovary, (2) to analyse whether there is an increase in ovule number per flower with increasing pollination unpredictability, and (3) to determine whether water availability could represent a counteracting direct or indirect force that better explains the observed variation in ovule number among populations. In a self-incompatible species, such as *E. coccineum*, stigmatic pollen counts can provide a misleading representation of effective pollination because of geitonogamous pollination. Therefore, we characterised pollination success by counting not only pollen grains received on the stigma (i.e. pollination quantity), but also number of pollen tubes growing in the style (i.e. pollination quantity and quality; Aizen and Harder 2007; Alonso *et al*. 2012). In summary, in the present study, we ask whether pollination unpredictability or abiotic factors (i.e. resource availability) better explains observed patterns of variation in ovule number per ovary across populations of a south-Andean Proteaceae.

Materials and methods

Study species and area

Embothrium coccineum is a widespread, self-incompatible shrub or small tree endemic to temperate forests of southern South America in Argentina and Chile, occurring from rainforests with >4000 mm of precipitation towards the west, to xeric ecotonal habitats with <700 mm of precipitation at the rain-shadowed eastern slopes of the Patagonian Andes. This abrupt environmental gradient relates to a size reduction of many different plant structures, including flowers and leaves from the wettest to the driest end (Chalcoff *et al*. 2008), but not to a decreasing number of flowers (Chalcoff 2008). Plants bloom from October to January and the protandrous, predominantly outcrossing flowers are red and tubular with four tepals fused with the anthers. As with other Proteaceae, the anthers dehisce before anthesis and the pollen is deposited onto a swollen part of the style, the 'pollen presenter', from which it is transferred to the pollinator's body. The stigma becomes receptive after pollen removal, and, thus, dichogamy prevents the saturation of the stigma with self pollen (for more details see Rovere and Chalcoff 2010).

Although flowers exhibit characteristics commonly associated with bird pollination (e.g. red, tube-like flowers; Faegri and van der Pijl 1979), the relatively voluminous nectar is readily accessible to most flower visitors because, as is typical of the Proteaceae, the tepals are not completely fused (Sleumer 1984). Therefore, although the hummingbird *Sephanoides sephaniodes* is the most efficient pollinator in populations where it is present, this plant species can be considered a pollinator generalist because their flowers are visited by a diverse assemblage of up to 32 different animal species (Chalcoff *et al*. 2012). This assemblage includes, besides the hummingbird, passerine birds, nemestrinid flies and several species of bees, principally the families Apidae and Colletidae (Smith-Ramírez and Armesto 1998, 2003; Smith-Ramírez *et al*. 2005; Devoto *et al*. 2006; Chalcoff *et al*. 2012).

Pollinator visitation, pollen receipt and ovule number

To determine the importance of most effective pollinators on pollination efficiency and predictability, we used visitation data previously reported in Chalcoff *et al*. (2012). There, we concluded that hummingbird, followed by nemestrinid flies and passerines birds, are the most effective pollinators, whereas bees act more as pollen thieves than pollinators. Therefore, we used the relative visit frequency of the former three considered together, to assess the influence of efficient pollinators on pollination predictability. In brief, visitation data were obtained from pollinator surveys of 16 populations of *E. coccineum* in the northern part of its distribution during the 2005–2006 flowering season (see Table S1, available as Supplementary Material, for population geographic location). In each population, we recorded flower visitors at focal branches or whole shrubs during 30-min censuses (i.e. one individual plant per census) between 0800 hours and 1800 hours throughout the flowering season, resulting in a total of 144 h of observation (16 populations × 18 periods per population × 0.5 h per census). From these data, we computed relative visitation frequency for each major pollinator taxon from absolute visitation rates estimated as number of visits per flower per 30 min (see Chalcoff *et al*. 2012).

In the same flowering season, we recorded flower visitors and from each of 10 randomly selected individuals in each population, we collected 20 pistils from flowers that had been open for several days and had started wilting. Each pistil was placed in individual 0.5-mL microcentrifuge tubes with formalin–alcohol–acetic acid (1 : 8 : 1, FAA). In the laboratory, ovules were counted directly in the dissected ovaries under a stereomicroscope. Then, styles were cleared in 10 mol L^{-1} NaOH, stained with 0.1% aniline blue in 0.1 mol L^{-1} K_3PO_4 (Martin 1959), squashed, and examined at ×400 using epifluorescence microscopy. We estimated total pollen receipt by counting both germinated and ungerminated pollen grains on the stigma. We also counted pollen tubes close to the basal end of the style (i.e. at about three-quarter distance down the length of the style). In this self-incompatible species, variation in the number of pollen tubes could be a more accurate predictor of pollination unpredictability than the variation in total pollen receipt. Specifically, in the Proteaceae, the style filters out self-incompatible pollen tubes, and, thus, the number of basal pollen tubes can be considered a proper estimator of cross-pollination (Fuss and Sedgley 1991). Also, pollen tubes may better reflect original pollen receipt than do pollen-grain counts because an undetermined fraction of pollen grains can become detached from stigmas in liquid-fixed pistils (Medan *et al*. 2013).

Because this species occurs along an abrupt west–east rainfall gradient (Rovere and Chalcoff 2010), water is probably the most limiting resource for growth and survivorship, particularly in the eastern-most populations of *E. coccineum*. We estimated the average annual precipitation at each population by using the Global Climate Worldclim GIS database (Hijmans *et al*. 2005), which provides climatic estimates with a resolution of ~1 km^2. Estimates from this database are consistent with data from weather stations and properly describe changes in rainfall in north-western Patagonia (K. Speziale, pers. comm.; for population precipitation data, see Table S1, available as Supplementary Material for this paper).

Statistical analysis

We estimated variance components in ovule number at different levels (i.e. within individuals, among individuals within populations and among populations) using hierarchical variance analysis. Then, we performed a hierarchical regression (Gelman and Hill 2007) with individual trees nested within populations, where we evaluated the association of pollination variability (i.e. unpredictability) with the relative visit frequency of efficient pollinators (i.e. hummingbirds, nemestrinid flies and passerine birds). We used the following three different measures of pollination variability: the standard deviations (s.d., accounting for the effect of mean pollination levels, i.e. pollen receipt and pollen tubes), coefficients of variation (CV) of the number of pollen grains deposited on the stigma and of pollen tubes growing in the style, and the proportion of flowers without pollen grains or pollen tubes, respectively (PFWPR and PFWPT hereafter). Using a similar hierarchical regression approach as above, we assessed the relationship of the (log-transformed) number of ovules per ovary for each individual, with pollination success (measured as a mean number of pollen grains in the stigma and pollen tubes in the style), pollination unpredictability, and rainfall across populations. All statistical analyses were conducted with the lme function of the nlme package in R ver. 2.11.1 (R Development Core Team 2013, Vienna, Austria).

In case of a significant effect of either pollination unpredictability or rainfall on ovule number, we analysed the geographical structuring of the residuals to certify that the proposed factor, rather than spatial autocorrelation, provided an adequate explanation for interpopulation variation in ovule packaging (see Diniz-Filho *et al*. 2003). To achieve this goal, we analysed the association between a matrix of geographical distances and a matrix of absolute mean residual differences by using a Mantel test with 9999 permutations (Sokal and Rohlf 1995), executed with the mantel.rtest function of the ade4 package (Dray and Dufour 2007), also in R ver. 2.11.1.

Results

Ovule packaging in *Embothrium coccineum* was highly variable, with the number of ovules per ovary varying between 3 and 20 (mean = 11.72, s.d. = 1.93, $n = 3062$). The ANOVA components showed a more or less equal partitioning of total variation among the different sources. Of all the variation, 34.4% occurred among populations, 27.1% among individuals within populations, and 39.5% within individuals. Thus, ~60% of the variation in ovule number per flower occurred at or above the individual level.

Pollination unpredictability also varied among populations, and, in general terms, populations at the most xeric sites exhibited lower means and higher unpredictability than populations at the wettest sites (see Fig. S1, available as Supplementary Material for this paper). The number of pollen grains received on the stigmas ranged (mean ± s.d.) from 4.8 ± 10.03 to 43.8 ± 39.8, whereas the number of pollen tubes growing in the style ranged from 0.1 ± 0.4 to 2.8 ± 3.9. Likewise, the s.d. ranged from 6.9 to 80.2 and the CV from 0.28 to 0.77 for pollen grains received, whereas the s.d. ranged from 0.41 to 5.3 and the CV from 0.45 to 1.14 for pollen tubes. In turn, the PFWPR varied from 2% to 36% and the PFWPT varied from 34.5% to 93.5%. Interestingly, only 3 of the 16 populations received, on average, fewer pollen grains on the stigmas than required to fertilise all ovules, but all the populations had, on average, fewer pollen tubes growing in the style than needed to guarantee maximum fertilisation.

Pollination success (i.e. means of both the number of pollen grains received and the number of pollen tubes) increased with the relative visit frequency of the most efficient pollinators (estimate ± s.e. = 0.23 ± 0.06, $t_{14} = 3.96$, $P = 0.001$; and estimate ± s.e. = 0.01 ± 0.004, $t_{14} = 3.4$, $P = 0.004$, respectively). Moreover, at least some of the measures of pollination unpredictability (i.e. PFWPR and PFWPT) increased with decreasing visitation by the most efficient pollinators (Table 1).

Table 1. Effect of relative visit frequency of the most efficient pollinators (hummingbirds, nemestrinid flies and passerine birds) on pollination unpredictability measured by standard deviations (s.d.) and coefficients of variation (CV) of both pollen receipt on stigmas and pollen tubes in styles, and the proportion of flowers without pollen grains or pollen tubes (PFWPR and PFWPT, respectively)

For the standard deviation, regression lines are based on partial regression coefficients after accounting for mean pollination levels (see Materials and methods). Significant *P*-values are highlighted in bold

Response variables	Effect	s.e.	*t* (d.f.)	*P*-value
s.d.$_{\text{pollen receipt}}$	–0.05	0.05	–0.1 (13)	0.9
s.d.$_{\text{pollen tubes}}$	–0.01	0.01	–1.9 (13)	0.08
CV$_{\text{pollen receipt}}$	0.001	0.001	1.3 (14)	0.2
CV$_{\text{pollen tubes}}$	–0.0001	0.001	–0.1 (14)	0.9
PFWPR	–0.15	0.1	–2.6 (14)	**0.02**
PFWPT	–0.21	0.1	–2.3 (14)	**0.04**

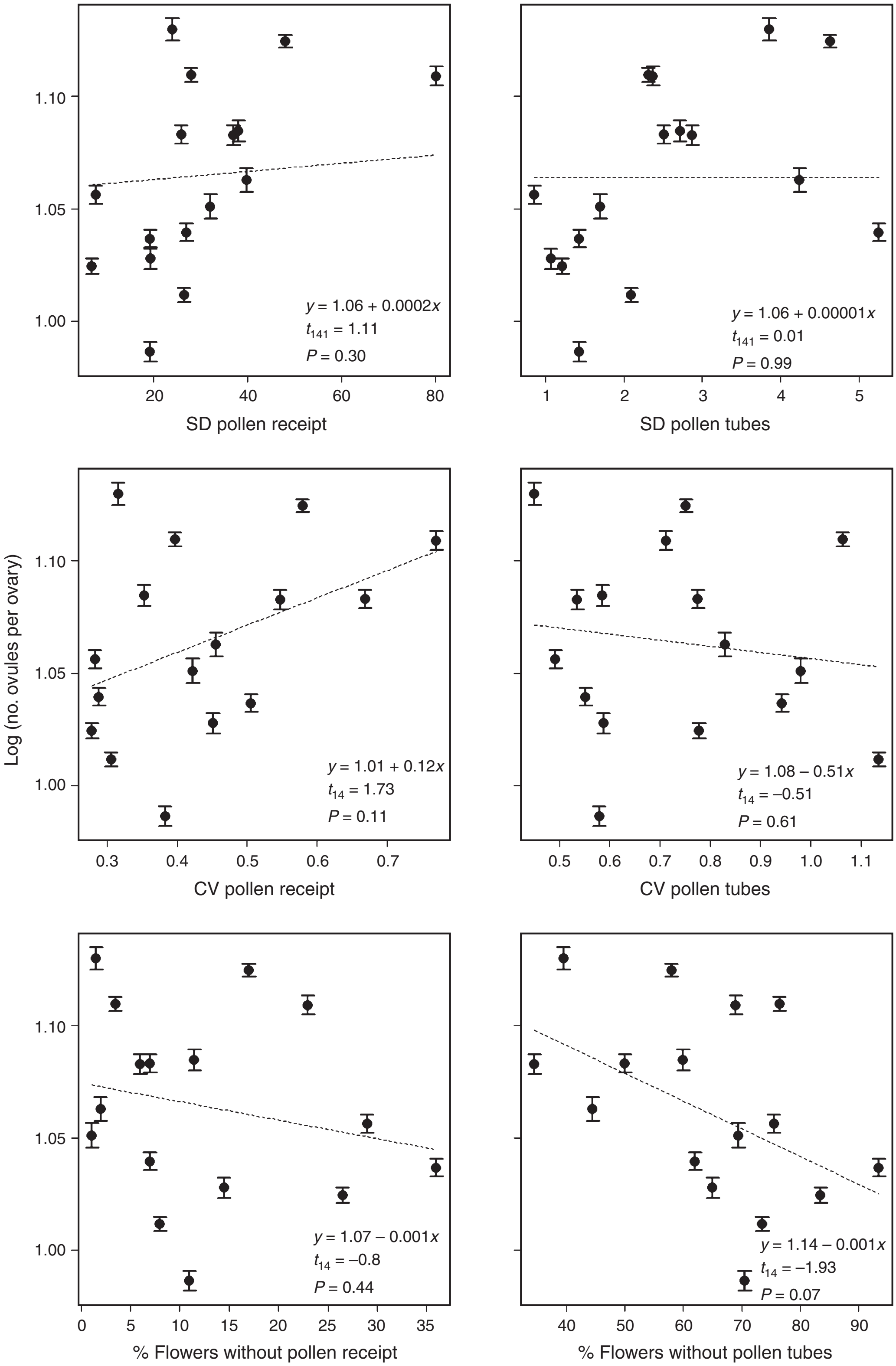

Fig. 1. Effects of pollination unpredictability, measured by the standard deviation (above) and coefficients of variation (middle) of both pollen receipt on stigmas and pollen tubes in styles, respectively, and the proportion of flowers without pollen grains or pollen tubes (below), on average (log-transformed) number of ovules per ovary (mean ± s.e.). The dashed regression lines and summary statistics indicate that pollination unpredictability does not provide an adequate explanation for variation in ovule number across populations of *Embothrium coccineum*. For the standard deviation, regression lines are based on partial regression coefficients, after accounting for mean pollination levels (see Materials and methods).

The observed relations between the different measures of pollination variability and ovule numbers did not support the hypothesis that a more unpredictable pollination environment selects for a higher number of ovules per flower. The (log) number of ovules did not show any significant association with the s.d. or CV of either the number of pollen grains received or the number of pollen tubes. Also, contrary to expectations, the number of ovules per flower did not show any relation with the PFWPR, and a marginally significant (but negative, rather than positive as expected) relation with PFWPT (Fig. 1). Also, in the case of partial regressions (Fig. 1, above panels), the mean number of pollen grains on the stigmas or tubes in the styles were not associated significantly with the average number of ovules per ovary across populations (estimate ± s.e. = 0.00002 ± 0.00003, $t_{2896} = 0.8$, $P = 0.42$ for the average number of pollen grains received; and estimate ± s.e. = 0.00006 ± 0.0003, $t_{2887} = 0.2$, $P = 0.84$ for the average number of pollen tubes). Thus, pollination-related factors did not seem to account for variation in ovule number.

Also, climatic conditions could affect ovule packaging directly through its effect on resource availability or indirectly via other life-history traits. The (log) number of ovules increased with increasing precipitation, with flowers from populations at the driest end of the rainfall gradient producing the smallest number of ovules (Fig. 2). Furthermore, when we added precipitation as a predictor in the above linear mixed-models testing the effect of pollination predictability, only precipitation had a positive effect on ovule number per ovary (Table 2). There was no evidence that neighbouring populations were more similar in the number of ovules than were more distant populations (Mantel test, $r = -0.003$, $P = 0.48$), after accounting for the effect of precipitation.

Discussion

Selection operates on heritable phenotypic variation occurring among individuals within populations, and, as a result, populations subjected to disparate selection pressures diverge (Endler 1986). As a consequence, our test is based on the implicit assumptions of existing variation in the number of ovules among individuals within populations, and also among populations. *Embothrium coccineum* complies with both assumptions; each of these two supra-individual levels accounted for ~30% of all variation in the number of ovules per ovary. Despite significant variation among populations in the number of ovules packaged per flower and that pollination unpredictability in *E. coccineum* tended to increase with decreasing visitation by the most efficient pollinator (i.e. hummingbirds, nemestrinid flies and passerine birds), the data revealed no evidence that pollination variability and ovule production were related. Instead, ovule number increased with increasing rainfall.

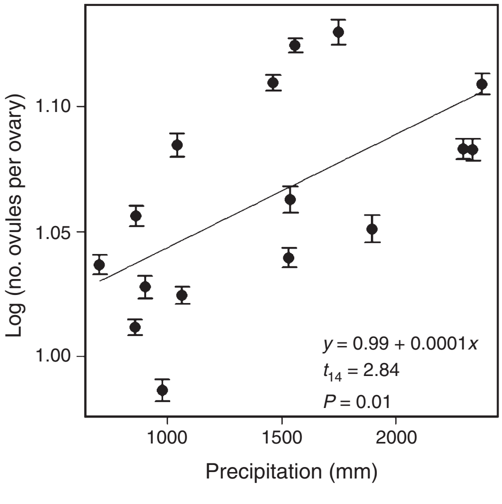

Fig. 2. Relation between (log) ovule number per ovary and mean annual precipitation across populations of *Embothrium coccineum*. Solid line and summary statistics indicate that ovule number increases significantly with rainfall. Regression coefficients were estimated using a hierarchical analysis (with individual trees nested within populations).

A pollinator assemblage dominated by inefficient pollinators should not only decrease mean pollination, but also pollination predictability (Garibaldi *et al*. 2011). This seems to be the case in *E. cocineum*, where the replacement of a bird- to a bee-dominated assemblage from west to east across north-western Patagonia not only depressed pollination but increased unpredictability in the occurrence of this mutualism. The outcome of this increasing stochasticity is an increasing proportion of flowers without pollen grains on their stigmas or pollen tubes in their styles with the loss of efficient pollinators towards the east. As a consequence, fruit set was increasingly limited by pollination eastward (Chalcoff *et al*. 2012).

Under this scenario of increasing pollination unpredictability, the bet-hedging hypothesis (Burd *et al*. 2009) would predict greater ovule production per flower to ensure that any flower that by chance received many pollen grains could make a significant contribution to overall plant seed production, a pattern validated by their among-species comparison (but see Schreiber *et al*. 2015). This should be particularly important in

Table 2. Partial effects of pollination unpredictability and rainfall across populations on (log-transformed) number of ovules per ovary

As measures of pollination unpredictability, we used standard deviations (s.d.) and coefficients of variation (CV) of both pollen receipt on stigmas and pollen tubes in styles, and the proportion of flowers without pollen grains or pollen tubes (PFWPR and PFWPT respectively). For the standard deviation, regression lines are based on partial regression coefficients after accounting for mean pollination levels (see Materials and methods). Significant *P*-values are highlighted in bold

Predictors	Effect	s.e.	*t* (d.f.)	*P*-value
$\text{s.d.}_{\text{pollen receipt}}$	0.0001	0.0002	0.82 (141)	0.4
Pollen receipt	0.00002	0.00003	0.8 (2896)	0.4
Precipitation	0.00004	0.00002	2.7 (14)	**0.02**
$\text{s.d.}_{\text{pollen tubes}}$	–0.0003	0.002	–0.15 (141)	0.9
Pollen tubes	0.00006	0.0003	0.2 (2887)	0.8
Precipitation	0.00005	0.00002	2.8 (14)	**0.01**
$\text{CV}_{\text{pollen receipt}}$	0.01	0.08	0.17 (13)	0.9
Precipitation	0.00004	0.00002	1.98 (13)	0.06
$\text{CV}_{\text{pollen tubes}}$	–0.01	0.04	–0.33 (13)	0.7
Precipitation	0.0001	0.00002	2.7 (13)	**0.02**
PFWPR	0.0003	0.001	0.29 (13)	0.8
Precipitation	0.0001	0.00002	2.59 (13)	**0.02**
PFWPT	–0.0002	0.0007	–0.27 (13)	0.8
Precipitation	0.0001	0.00002	2.17 (13)	**0.05**

a strongly pollination-limited species such as *Embothrium coccineum*, where any lost of pollination opportunity should affect female fitness directly (Ashman *et al*. 2004). Nevertheless, pollination unpredictability estimated from counting pollen grains on the stigma, which reflects stochasticity in pollination quantity, or pollen tubes in the style, which reflects stochasticity in both pollination quantity and quality, did not relate positively to ovule number, and, thus, our data for this particular species are not supportive of this bet-hedging hypothesis.

Instead, ovule number increased with rainfall, suggesting that abiotic factors could influence how many ovules can be packaged within an ovary. To our knowledge, this is the first empirical evidence in support of the alternative hypothesis that variation in ovule number can be determined by the environment. Specifically, increasing drought could not only limit the size and number of different organs, e.g. leaves (Dudley 1996), but also number of ovules. However, interpreting the role of drought is complex because plants can respond to this factor both plastically and evolutionarily. Furthermore, drought as a selection factor can affect ovule number, its selection target, directly or indirectly. Individuals of *E. coccineum* growing in wet environments have larger flowers and leaves than do individuals growing in dry environments (Chalcoff *et al*. 2008), and plants growing in a common environment seem to maintain these characteristics (M. A. Aizen, pers. obs.). One plausible hypothesis is that drought could be selecting for smaller leaves to avoid excessive water loss through evapotranspiration (Dudley 1996). Thus, a reduction in flower parts, including a decrease in ovule number associated with smaller ovaries, would represent an indirect correlated change associated with leaf-size reduction (see Chalcoff *et al*. 2008). Indeed, it has been observed that ovule number per ovary is correlated with flower size across and within species (Cruden 2000, and references therein). However, a controlled common garden experiment would be necessary to address the role of direct and correlated plasticity and selection in determining ovule number.

Finally, as we mentioned previously, pollination is a spatially and temporally unpredictable process (Herrera 1988), so the stochasticity in pollination may vary not only among populations but also among reproductive seasons. According to sexual selection theory, female lifetime reproductive success can be more limited by resources rather than by pollen receipt, even though reproduction can be limited by pollination in any single season (Ashman *et al*. 2004, and references therein). Therefore, under temporal pollination unpredictability, a long-lived species with multiple mating opportunities over its lifetime, such as *Embothrium coccineun*, might not be under strong selection to increase ovule number. This might explain the lack of support for the bet-hedging hypothesis, as was found here. However, many of the species included in the comparative analysis by Burd *et al*. (2009) were perennial, and it has been shown that species that are pollen-limited during most reproductive seasons are also pollen-limited over their lifespan (Calvo and Horvitz 1990). Therefore, it is unlikely that this proposal could explain the lack of support we found in favour of the bet-hedging hypothesis.

Existing variability in ovule number within individuals (~40% of all variation) also indicates that ovule number might be affected directly by environmental factors, ontogeny, as well as the resource status of each branch, all being factors that might be in part associated with architectural effects (Herrera 2009, and references therein). Thus, reduction in ovule number above the individual level could be interpreted at least in part as a phenotypically plastic response to the prevailing environmental conditions. Most likely, both genetic and non-genetic determinants underlie variation in ovule packaging within and among populations.

In conclusion, here we found that the number of ovules packaged in the ovaries of *E. coccineum* is a highly variable trait, with a large fraction of this variation occurring among populations. Nevertheless, the pollination bet-hedging hypothesis, i.e. selection for increasing number of ovules per flower should become stronger in populations experiencing more unpredictable pollination (Burd *et al*. 2009), does not provide an adequate explanation for divergence in ovule number in this species. Instead, decreasing resource availability (i.e. increasing drought) could directly and/or indirectly limit the maximum number of ovules that could be packaged within the ovary. Therefore, in species growing under contrasting environmental conditions, resources could be a more powerful factor limiting the number of gametes than is pollination.

Supplementary material

Supplementary material with the locations, geographical coordinates and average annual precipitations of the 16 populations studied of *Embothrium coccineum* (Table S1), and the relationship between pollination unpredictability and precipitation (Fig. S1) are available at the Journal's website.

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Supplementary Material

Pollination unpredictability and ovule number in a South-Andean Proteaceae along a rainfall gradient

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Table S1. **Locations and details of the 16 populations studied of *Embothrium coccineum*.** Ref. = reference number for figure below, Name =name of the population, Lat (S) = south latitude, Long (W) = west longitude, Elev. = elevation (meters asl), Prec. = average annual precipitation (mm; obtained from the Global Climate Worldclim GIS). Table and figure partially taken from Chalcoff *et al.* 2012.

Ref.	Name	Lat (S)	Long (W)	Elev.	Prec.	Country
1	Aduana	40º 40	71º 44	893	1539	Argentina
2	Aeropuerto Bariloche	41º 07	71º 13	828	863	Argentina
3	Puerto Blest	41º 03	71º 49	780	1533	Argentina
4	Circunvalación	41º 09	71º 15	888	858	Argentina
5	Cordillera de la Costa	40° 37	73º 25	332	1750	Chile
6	Entrelagos	40º 37	72º 48	129	1561	Chile
7	Lago Espejo	40º 39	71º 42	860	1464	Argentina
8	Estación Biológica	41º 53	73º 40	12.1	2298	Chile
9	Gutiérrez	41º 10	71º 25	1058	978	Argentina
10	Laguna Juncos	41º 03	71º 02	995	702	Argentina
11	Manso	41º 34	71º 46	460	1898	Argentina
12	Cº Otto	41º 09	71º 19	900	902	Argentina
13	Paraguay	41° 07	73 º 10	300	2340	Chile
14	Puyehue	40º 40	72º 09	460	2382	Chile
15	Lago Steffen	41º 31	71º 28	928	1043	Argentina
16	Colonia Suiza	41º 08	71º 26	878	1065	Argentina

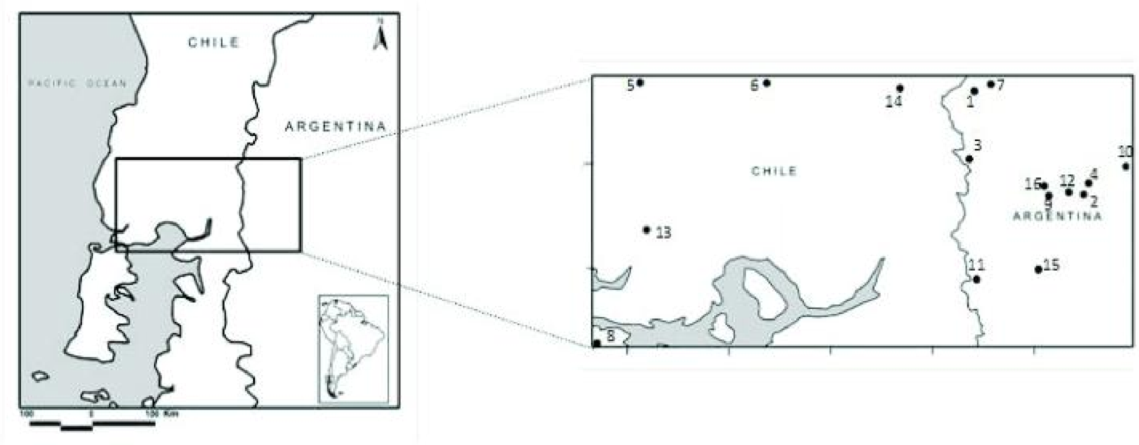

Fig. S1. Effects of mean annual precipitation across populations of *Embothrium coccineum* on pollination unpredictability, measured by the standard deviation (above) and coefficients of variation (middle) of both pollen receipt on stigmas and pollen tubes in styles respectively, and the proportion of flowers without pollen grains or pollen tubes (below). The summary statistics indicate that although rainfall generally does not provide an adequate explanation for pollination unpredictability, in general terms the unpredictability decrease with precipitation. For the standard deviation, regression lines are based on partial regression coefficients after accounting for mean pollination levels (see Materials and Methods).