Received: 1 November 2019 | Accepted: 11 April 2020

DOI: 10.1111/1365-2435.13581

RESEARCH ARTICLE

Functional Ecology BRITISH ECOLOGICAL SOCIETY

To bee or not to bee: The 'raison d'être' of toxic secondary compounds in the pollen of Boraginaceae

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Funding information
University of Neuchatel; Foundation Joachim de Giacomi

Handling Editor: Diane Campbell

Abstract

1. While the presence of secondary compounds in floral nectar has received considerable attention, much less is known about the ecological significance and evolutionary origin of secondary 'toxic' compounds in pollen. It is unclear whether the presence of these compounds in pollen is non-adaptive and due to physiological 'spillover' from other floral tissues, or whether these compounds serve an adaptive function related to plant–pollinator interactions, such as protection of pollen against pollen thieves.
2. Combining an experimental approach with phylogenetic comparative methods, and using western Palaearctic Boraginaceae as a model system, we investigate how pollen secondary metabolites influence, and are influenced by, relationships with bees, the main functional group of pollen-foraging pollinators.
3. We found a significant relationship between the levels of secondary compounds in the corollas and those in the pollen in the investigated species of Boraginaceae, suggesting that baseline levels of pollen secondary compounds may partly be due to spillover from floral tissues. At realistic levels, pollen secondary compounds showed significant detrimental effects on bee pre-imaginal development, in agreement with previous egg-transfer experiments showing that in some cases Boraginaceae pollen did not support pre-imaginal development in bees not specialized on these plants.
4. We also show that phylogenetically independent Boraginaceae taxa rewarding pollinators with pollen in addition to nectar exhibit significantly lower levels of toxic compounds in the pollen than taxa where the main reward is postulated to be nectar. Lastly, in contrast to our predictions, there was no positive association between toxin levels in the pollen of a given plant taxon and the number of bee species specialized on this taxon.
5. We integrate all these findings and formulate an evolutionary scenario to account for the presence of toxic compounds in the pollen of Boraginaceae. We suggest that baseline levels of toxic compounds may be found in pollen due to spillover from other floral tissues and not primarily because of bee–flower interactions. Since pollen toxins can have detrimental effects on bees, we propose that

selection acts to lower pollen toxin levels in plants where pollen, in addition to nectar, serves as a reward to bees.

KEYWORDS

alkaloid, floral chemistry, floral reward, herbivory, optimal defence theory, plant–insect interactions, pollination, secondary metabolites

1 | INTRODUCTION

The presence of toxic secondary metabolites in floral rewards—nectar and pollen—and their potential effects on pollinators remain a major enigma in pollination ecology (Irwin, Cook, Richardson, Manson, & Gardner, 2014; Stevenson, Nicolson, & Wright, 2017). While numerous studies have examined the ecological significance of nectar toxins, less is known on pollen secondary compounds (Rivest & Forrest, 2020). Yet pollen, in contrast to nectar, often contains high levels of secondary compounds (Palmer-Young et al., 2019), with concentrations that are comparable to or even higher than those in vegetative tissues (Gosselin et al., 2013). Consequently, these compounds may strongly impact the interactions between plants and pollinators.

From the plant perspective, three main hypotheses have recently been developed to account for the presence of toxic secondary compounds in pollen (Rivest & Forrest, 2020). The pleiotropy (or 'non-adaptive') hypothesis suggests that the presence of toxic compounds in the pollen is explained by physiological spillover (or passive leakage: see Manson, Rasmann, Halitschke, Thomson, & Agrawal, 2012) from nearby tissues or vascular fluids. According to this hypothesis, toxic compounds are found in floral rewards as an indirect consequence of the accumulation of these compounds (or their precursors: see Hartmann, 1999) in other plant tissues such as the developing flower buds. Second, the defence-against-pollen-collection hypothesis points to an adaptive role of these compounds against pollen-collecting visitors which do not, or only poorly, contribute to pollen transfer. Third, pollen secondary compounds may function as antimicrobials against pollen-colonizing micro-organisms (Rivest & Forrest, 2020). Empirical evidence for these hypotheses is so far limited. Dissimilarities in the secondary compound composition among floral tissues (e.g. Cook, Manson, Gardner, Welch, & Irwin, 2013) and lack of correlation between the amounts of compounds detected in pollen and in other floral tissues (Heiling, Cook, Lee, & Irwin, 2019) suggest that pleiotropy is not generally a sufficient explanation to account for the presence of defence compounds in the pollen (Rivest & Forrest, 2020). However, a comprehensive test of the pleiotropy hypothesis within a phylogenetic framework is so far lacking for pollen compounds (see Manson et al., 2012 for nectar secondary compounds). Similarly, numerous studies have suggested that pollen secondary compounds may protect pollen against pollen thieves (e.g. Bukovinszky et al., 2017; Haider, Dorn, & Müller, 2013; Haider, Müller, & Dorn, 2014; Praz, Müller, & Dorn, 2008a; Sedivy, Müller, & Dorn, 2011), but conclusive evidence is so far lacking (Sedivy, Piskorski, Müller, & Dorn, 2012). Recently, Wang, Tang, Wu, Wu, and Huang (2019) provided evidence that high levels of chemical defences in the pollen impacted the behaviour of pollinators, thereby enhancing pollen transfer, but the universality of this phenomenon remains to be investigated.

From the pollinator's perspective, the impact of realistic levels of pollen secondary compounds on the behaviour and reproduction success of pollen-feeding pollinators remains poorly explored. At realistic concentrations, pollen secondary compounds have been shown to impact larval development in bumblebees (Arnold, Idrovo, Arias, Belmain, & Stevenson, 2014) and in honeybees (Lucchetti, Kilchenmann, Glauser, Praz, & Kast, 2018), and to modulate foraging preferences of bumblebees (Wang et al., 2019). All these studies have been performed using eusocial bees, which develop in colonies, are largely generalist in their pollen choices, mix various sources of pollen as larval food, and, in the case of honeybees, consume pollen mostly in the adult stage and feed their larvae with nursing secretions. By contrast, incidence of floral specialization is high in solitary bees (Danforth, Minckley, & Neff, 2019), which often forage on a restricted source of pollen (oligolectic bees). The impact of pollen secondary compounds on solitary bees is largely unknown (Sedivy et al., 2012). If these compounds, at realistic concentrations, have the potential to hamper larval development, they may impose physiological constraints on host-plant choices in bees. Moreover, under this scenario solitary bees would need to evolve specific counter-adaptations to deal with pollen toxins, and this could result in the phylogenetic conservatism observed in host-plant use by bees (Sedivy, Praz, Müller, Widmer, & Dorn, 2008) or in the high incidence of specialization in solitary bees. For instance, flowers of the genus *Aconitum* bear particularly high amounts of alkaloids in the pollen (Gosselin et al., 2013) and are the exclusive pollen hosts of the only two known specialist bumblebees, *Bombus consobrinus* and *Bombus gerstaeckeri* (Goulson, 2003). Bees may also specialize on plants producing toxic pollen to reduce their pathogen or cleptoparasite loads (LoCascio, Aguirre, Irwin, & Adler, 2019; Spear, Silverman, & Forrest, 2016). These hypotheses remain largely untested in solitary bees.

We here address the evolutionary origin of toxic pollen and examine its impact on bee–flower interactions using the plant family Boraginaceae as a model system. Boraginaceae represents an important source of pollen and nectar for specialist and generalist bees (Müller, 2019; Sedivy, Dorn, Widmer, & Müller, 2013;

Westrich, 1989), and exhibit three main floral morphological types, which reflect divergent pollination modes (Sedivy et al., 2013): the *Pulmonaria*-, *Echium*- and *Borago*-types (Figure 1; see Table S1 for a detailed review of the morphology and pollination biology of these three floral types). Current evidence based on the frequency, behaviour and efficiency of floral visitors suggests that the primary reward in species of the *Pulmonaria*- and *Echium*-types is nectar (see in particular Ferrero, Castro, Sánchez, & Navarro, 2011). In contrast, the *Borago*-type species display enlarged anthers that are united medially to form a pollen-dispensing cone, similar to the structure observed in solanoid flowers ('scatter cone flowers'; Faegri, 1986; Vogel, 1996). Species of the *Borago*-type have pendulous flowers, which are hardly visited by non-bee visitors such as Diptera or Lepidoptera. Female bees collect both pollen and nectar from such flowers, and often sonicate the flower to gather the pollen (Sedivy et al., 2013; Teppner, 2011). Pollen is thus considered to constitute a reward in addition to nectar in *Borago*-type flowers. Current phylogenetic hypotheses for Boraginaceae suggest multiple independent origins of the pollen-dispensing morphology observed in the *Borago*-type (Cohen, 2014).

Plants in the Boraginaceae family contain toxic pyrrolizidine alkaloids (hereafter called PAs) in the roots, the leaves and often also in the floral parts (El-Shazly & Wink, 2014) including in pollen and nectar (Lucchetti et al., 2016). While most of the literature on PA toxicity has focused on their role against leaf and root feeders (Hartmann, 1999; Macel, 2011), previous work has suggested that PAs can also be toxic to pollen feeders (Lucchetti et al., 2018). Provisions of pollen and nectar of *Echium vulgare* were shown to be unsuitable for larvae of several wild bee species not specialized on this plant genus (Praz et al., 2008a; Sedivy et al., 2011). *Echium vulgare* pollen contains high levels of amino acids (Somerville & Nicol, 2006), but also particularly high levels of PAs (Lucchetti et al., 2016), suggesting that PAs, and not lack of nutrients, underlie the observed mortality of the larvae, although the direct toxicity of PAs for solitary bees has not been tested so far.

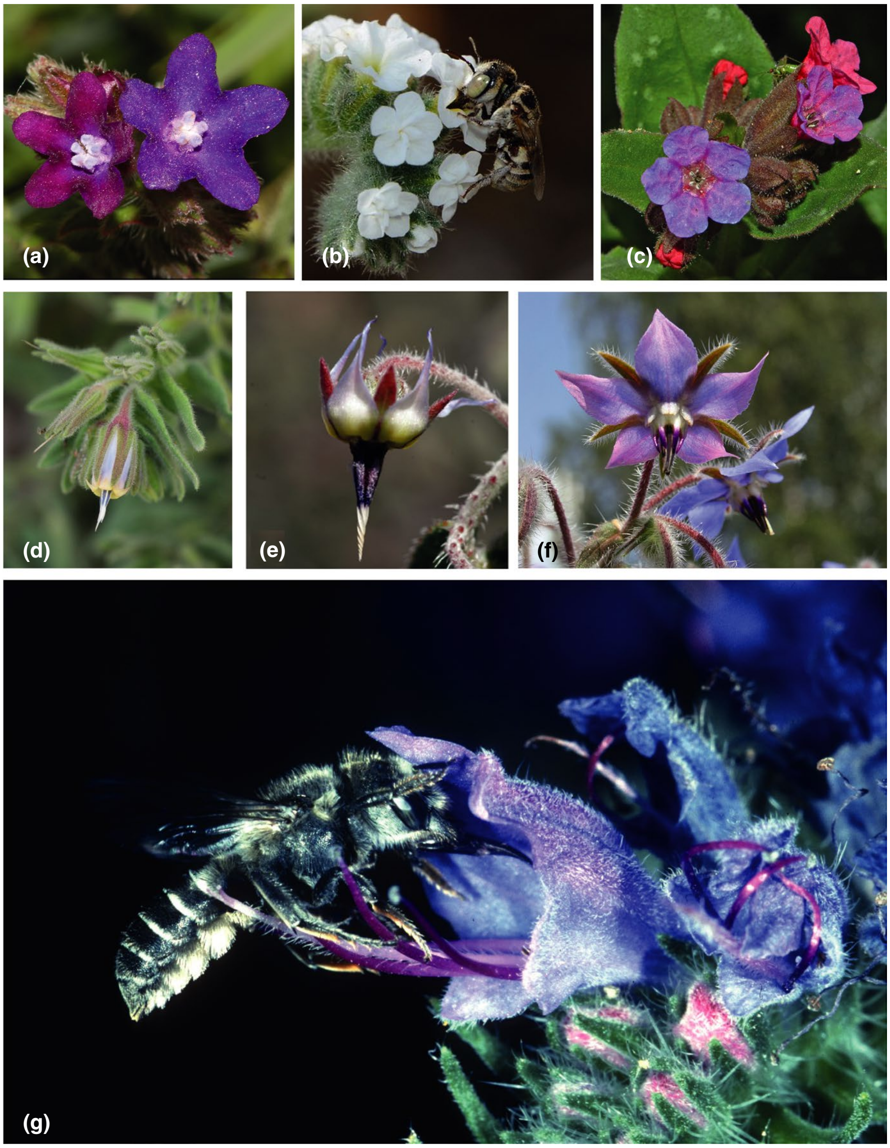

FIGURE 1 Variability of floral morphology in the three floral types of Boraginaceae. (a–c) *Pulmonaria*-type, with narrow, erect corolla and hidden anthers; (a) *Anchusa officinalis*; (b) *Heliotropium* sp. with the oligolectic bee *Haetosmia vechti*; (c) *Pulmonaria officinalis*; in *Anchusa* (a), access to the anthers is further restricted by the presence of hairy scales. (d–f) *Borago*-type, with pendulous flowers and enlarged anthers joined medially to form a scatter cone; the three presented genera represent three phylogenetically independent origins of this floral morphology; (d) *Podonosma orientalis*; (e) *Trichodesma africana*; (f) *Borago officinalis*. (g) *Echium*-type, with zygomorphic corolla that opens laterally and exposed anthers and style; the figure presents *Echium vulgare* visited by the oligolectic bee *Hoplitis adunca*. Credits: W. Obermayer (a), G. Pisanty (b), E. Balocchi, Wikimedia Commons (c), D. Bénon and V. Trunz (d), A. Müller (e, g), Paasikivi, Wikimedia Commons (f)

We quantified PAs in the pollen and in the corolla of selected members of Boraginaceae. Levels of PAs in the corollas were used as a proxy for the average amount of PAs present in the inflorescence. By supplementing pollen provisions with realistic amounts of PAs isolated from one exemplary Boraginaceae species, we next examined the effects of PAs on the development of solitary bee larvae. Specifically, we asked the following questions: (a) Is there a correlation between the concentrations of PAs in the corolla and in pollen across species? (b) At realistic concentrations, do pollen PAs have the potential to induce fitness costs in solitary bees by hampering their pre-imaginal development? (c) Is there a difference in pollen PA concentration between plants rewarding their pollinators with pollen and those primarily rewarding pollinators with nectar only? And (d) is there a difference in pollen PA concentration between plants hosting pollen-specialist bees and those primarily hosting generalists? We predict that: (a) pollen PAs positively correlate with PAs in the corolla, suggesting that the presence of PAs in pollen is at least partly due to physiological spillover from other floral tissues (hypothesis 1); (b) PAs of *E. vulgare* are toxic to solitary bee larvae not specialized on this plant genus when supplemented to their host pollen provisions (hypothesis 2); (c) Boraginaceae species rewarding their pollinators with both pollen and nectar (*Borago*-type) exhibit lower PA contents in pollen than those rewarding pollinators primarily with nectar (*Pulmonaria*- and *Echium*-types; hypothesis 3); (d) There will be a positive association between PA levels in the pollen of a given plant taxon and the number of specialist bee species on this taxon (hypothesis 4).

2 | MATERIALS AND METHODS

2.1 | Plant species selection

To address our hypotheses, we selected 26 species of Boraginaceae present in temperate Europe and in the Mediterranean region, representing 16 genera (Table 1; Table S1). Our sampling specifically included several species within species-rich genera, representing the three floral morphological types outlined in the introduction (twelve, nine and five species for the *Pulmonaria*-, *Borago*- and *Echium*-types respectively). Our species selection includes five phylogenetically independent origins of the *Borago*-type morphology (Cohen, 2014).

2.2 | Floral visitors and pollination biology

We compiled the information available on the pollination biology of the 26 investigated species, including the total spectrum of visitors (specifically the importance of bees compared to all visitors), observations on nectar- or pollen-collecting behaviours, as well as a list of specialist bees (Table S1). With respect to visits by bees, distinction has rarely been made between nectar visits and pollen visits (but see for example Corbet, 1978; Müller, 1995; Teppner, 2011). To test the hypothesis that pollen constitutes a reward in *Borago*-type species, but not in *Echium*, we compared the ratio of nectar-to-pollen visits by bumblebees in large, co-occurring populations of three species, *Cerinthe minor*, *Borago officinalis* (both of the *Borago*-type) and *Echium vulgare*, at the Botanical Garden of Neuchatel, Switzerland. Visitors to the three plant species were surveyed in 1-m plots in 10-min sessions, on three different days; sessions alternated between the three plant species (see Supporting Information for details). While we acknowledge that the survey of three species in one unique locality provides limited evidence to be generalizable across all Boraginaceae, this survey complements previous work done on other species, and especially allows simultaneous comparisons of the behaviour of floral visitors on exemplary Boraginaceae species having distinct floral morphologies.

Our chemical analyses suggest high variation in the levels of PAs in the pollen of different species of the genus *Echium* (see Section 3). To explore the relationship between pollen toxicity and the spectrum of visitors and bee behaviour in this genus, we surveyed visitors in one species with low PA levels, *E. wildpretii* on the Island of Tenerife (Teide National Parc), and one species with high PA levels, *E. vulgare* in a natural habitat in southern Switzerland (see Supporting Information for details on these surveys). Visitors to both plant species were surveyed in 10-min sessions, each on two different days; for the large-sized *E. wildpretii*, one focal plant was surveyed during each session, and a new plant was selected for subsequent sessions, for a total of approximately 40 plant individuals. For the smaller *E. vulgare*, which often grows in tangled patches of several individuals, observations were made in two 1-m plots (one different plot per day) containing numerous flowers corresponding to several plant individuals (approximately two to five). While it would be desirable to expand these observations to other species of the diverse genus *Echium*, we only found small populations of the other species investigated here and their visitors could therefore not be surveyed.

2.3 | PA profiling in pollen and corollas

For each of the 26 species studied, we hand-collected a minimum of 1 mg of pollen using tweezers; the pollen was lyophilized and stored in microcentrifuge tubes at −80°C until chromatographic analyses. For all species (with the exception of two missing values), we also sampled a minimum of 5 mg of corolla, which were placed into an open vial, dried in the field using silica gel and stored at −80°C. Details on the chemical analyses (following Lucchetti et al., 2016) are given in the Supporting Information. Pollen was sampled from numerous plant individuals (up to several dozens for minute flowers) to obtain enough pollen for analysis. Whenever possible, we sampled three times 1 mg of pollen either from three different plant populations or from three plant patches from the same population; corollas were sampled from one randomly selected plant from the same patches. For rare species, only one pollen sample could be collected (Table 1; Table S2). We summed all PAs found in each plant tissue and did not explore the

TABLE 1 The 26 species of Boraginaceae sampled in this study, classified into three floral morphological types, with their geographic range, the levels of PAs measured in the pollen and corollas (in μg/mg), and the number of specialist bee species on each genus (except for *Anchusa*; see text). Missing values are indicated by 'NA'

Type	Name	Distribution	No. of samples	PA concentrations in the pollen	PA concentrations in corollas	No. of specialist bee species (one value per genus except for *Anchusa*)
Pulmonaria-type	*Anchusa azurea*	Mediterranean region	3	3.168	17.962	0
	Anchusa officinalis	Mediterranean region, temperate Europe	2	1.799	0.498	3
	Buglossoides purpurocaeruleum	Southern and central Europe	1	0.004	0.296	0
	Cynoglossum montanum	Eastern Mediterranean region	1	0.136	41.673	1
	Cynoglossum officinale	Temperate Eurasia	1	2.251	64.758	
	Glandora oleifolia	Western Mediterranean region	1	0.990	4.301	0
	Heliotropium arbainense	Saharo-Arabian region	1	4.976	72.519	5
	Lithodora fruticosa	Western Mediterranean region	2	0.702	1.443	2
	Moltkia suffruticosa	Mediterranean region	1	0.339	8.844	0
	Myosotis sylvatica	Temperate Europe	1	3.281	2.773	0
	Nonea erecta	Eastern Mediterranean region	1	0.059	NA	1
	Pulmonaria obscura	Temperate Europe	3	0.003	0.085	0
Borago-type	*Borago officinalis*	Temperate Europe (originally presumably Northern African)	2	0.027	0.019	0
	Cerinthe glabra	Temperate Europe	2	0.905	5.128	2
	Cerinthe major	Mediterranean region	1	0.350	13.989	
	Cerinthe minor	Mediterranean region	1	0.129	4.454	
	Onosma helvetica	Mediterranean region	3	0.185	6.481	5
	Onosma sericea	Eastern Mediterranean region	1	0.018	NA	
	Podonosma orientalis	Eastern Mediterranean region	3	0.185	1.964	1
	Symphytum officinale	Temperate Europe	1	0.003	0.155	1
	Trichodesma africana	Saharo-Arabian region	3	0.019	1.708	2
Echium-type	*Echium aculeatum*	Canary Islands	3	0.023	10.218	12
	Echium bonnetii	Canary Islands	1	2.143	18.199	
	Echium virescens	Canary Islands	1	0.393	2.849	
	Echium vulgare	Temperate Europe	3	4.678	29.140	
	Echium wildpretii	Canary Islands	3	0.121	26.947	

qualitative differences in PA composition among species or tissues because of the difficulties in precisely characterizing the different compounds, their reduced form and possible isomers (note that, by contrast, the identification of PAs as such was always straight forward). Moreover, previous research has shown that each plant tissue may have a distinctive bouquet of PAs, which is often composed of structurally close molecules (Hartmann, 1999; Lucchetti et al., 2016) and which has been suggested to be derived from a common alkaloid intermediate (Hartmann, 1999). We restricted our analyses to PAs and did not include other secondary metabolites, which have been reported in the pollen and nectar of some Boraginaceae (e.g. spermidines and flavonoids;

Palmer-Young et al., 2019); unlike PAs some of these metabolites are involved in primary plant functions such as pollen germination or pollen tube formation (Aloisi, Cai, Serafini-Fracassini, & Del Duca, 2016), or were suggested to act as antioxidants or protective compounds against UV radiation (Taylor & Grotewold, 2005), rather than as defence compounds against herbivores. Finally, we would like to emphasize that our sampling protocol favoured a high number of species over replication within species. While the low level of replication within species is a limitation of our study, it is often unavoidable when performing species-level comparative studies. In addition, the pooling of pollen samples over different plant individuals was necessary to obtain sufficient quantities of pollen. Similar pooling was not achieved for the samples of corollas, which could result in higher variance for corollas than for pollen. Overall, our results suggest limited variation within plant species in those for which more than one sample was analysed (Table S2), especially for key taxa such as *Echium vulgare*, *E. wildpretii* and *Pulmonaria obscura*.

2.4 | Effect of pollen PAs on bee larval development

To address the potential toxicity of PAs on bees, we isolated the three main PAs found in the pollen of *Echium vulgare* (echimidine, echivulgarine and acetylechimidine; Lucchetti et al., 2016) from 1,550 g of inflorescence of this plant as described in Lucchetti et al. (2018). We then specifically tested whether pollen PAs in *E. vulgare* account for the previously documented failure of two solitary bee species (*Chelostoma rapunculi* and *Osmia bicornis*; Praz et al., 2008a; Sedivy et al., 2011) to complete larval development on pure *Echium* pollen and nectar provisions. We also included a third species closely related to *O. bicornis*, *O. cornuta*, which was shown to be able to develop on pure *Echium* provisions (Sedivy et al., 2011). It is noteworthy that none of these three osmiine species regularly collect pollen from *Echium*. We supplemented natural pollen and nectar provisions of these three bee species with realistic levels of PAs found in pure *Echium* pollen provision, as quantified from three cells from nests of the *Echium* specialist *Hoplitis adunca*. We used artificial cells made of drilled wood, following Sedivy et al. (2011; see Supporting Information for additional details). Larvae were checked every 3–4 days during larval development; we registered whether larvae were alive, dead and whether cocoon spinning was initiated. After overwintering (see Supporting Information), cocoons were opened to assess whether the imago was fully developed and alive, in which case adults were sexed and weighed.

2.5 | Statistical analyses

Fourth-root transformation was applied to PA values to correct for non-normality for all statistical tests (after transformation, $p > 0.05$ for all groups tested; Shapiro–Wilk normality tests). To examine the correlation between corolla and pollen PAs (hypothesis 1), we first inferred a phylogenetic tree based on published sequences (Cohen, 2014) using maximum likelihood in RAxML (Stamatakis, 2014). Next, we performed a regression analysis between the average values of PAs in corolla and pollen for each species, while taking into account the phylogenetic relationship among species using a Brownian motion model for trait evolution (Phylogenetic Generalized Least Square, or PGLS; *pgls* function in the package CAPER; Orme et al., 2013) in R (R Core Team, 2017). The λ parameter, which predicts the pattern of covariance among species for a given trait, was estimated by maximum likelihood (ML) to avoid inflating type I errors. Phylogenetic signal was estimated using kappa statistics as implemented in the package PHYTOOLS (Revell, 2012).

Larval survival across PA treatments (hypothesis 2) was estimated using Kaplan–Meier statistics and the proportion of living adults using chi-squared tests (package SURVIVAL; Therneau & Lumley, 2019). To investigate whether the levels of PAs in the pollen differ with plant pollination biology (hypothesis 3), we compared PA levels in pollen and in corollas across two groups of plant species, those rewarding pollinators with pollen and nectar (*Borago*-type) versus those where nectar is postulated to be the main reward (*Echium*- and *Pulmonaria*-types), using PGLS. As above, the λ parameter was estimated by ML. As an additional piece of evidence for evaluating the extent to which selection was acting on PA levels, we also compared continuous trait evolution models under Brownian motion (BM) or Ornstein–Uhlenbeck (OU) processes for PA levels in corollas and in pollen. A BM process suggests that a continuous trait evolves randomly across the phylogeny (pure drift model), while OU incorporates both drift and selection (Butler & King, 2004). In addition, multiple evolutionary optima (OUM models) may be incorporated into OU models if selection is hypothesized to act differently in two or more groups (Butler & King, 2004); groups were the same as those examined in PGLS analyses (*Borago*-type species vs. other types) and the same phylogenetic tree was used. We used the package MVMORPH (Clavel, Escarguel, & Merceron, 2015) to evaluate these models in R and selected among alternative hypotheses using corrected Akaike information criterion values (hereafter AICc) and, for nested models, the likelihood ratio test implemented in MVMORPH.

Lastly, to examine the link between PA concentrations in pollen and floral specialization in bees (hypothesis 4), we reduced our dataset to 16 Boraginaceae genera by averaging PA levels within each genus, since bees typically specialize at the genus level. An exception was made for both species of *Anchusa*, which were kept as two species, since the known oligolectic bees on *A. officinalis* do not appear to visit *A. azurea* (Westrich, 1989; personal observations). We first examined whether PA levels in pollen and in corollas differ between groups (plants hosting specialist bees vs. the others) using PGLS analyses; we then also examined whether there was a relationship between PA concentrations in the pollen and the number of specialist bee species per plant genus using PGLS analyses. The phylogenetic tree used was obtained by pruning the tree reconstructed above. For the PGLS analysis with groups, the λ parameter could not be estimated using ML; we thus successively fixed it to 0.1 and 1.0. The number of specialist bee species per Boraginaceae taxa was log-transformed (1 was added to avoid zero values) to correct for non-normality.

3 | RESULTS

3.1 | Pollination biology of the three floral types

The spectrum of visitors and the pollination biology of each species examined are detailed in Table S1. Bees regularly gather both pollen and nectar from all three types of Boraginaceae, and species of solitary bees may specialize on Boraginaceae genera from all three types. *Echium* was the genus that was associated with the highest number of specialized bees (12 species), followed by *Heliotropium* and *Onosma* (five species) and *Anchusa officinalis* (three species; Table 1).

In the site where *Echium vulgare*, *Cerinthe minor* and *Borago officinalis* co-occurred, our survey of visitors indicated that 77.8% of the bumblebee visits on *E. vulgare* were for nectar only, while *C. minor* and *B. officinalis* (both in the *Borago*-type) were mostly visited for pollen (80.9 and 97.6% of visits respectively; Table S3). The *Echium* species with low PA levels in the pollen, *E. wildpretii*, was predominantly visited by bees (99.3%); among bees 30.0% of the visits were by the only native bumblebee species (*Bombus terrestris canariensis*) and 70.0% by solitary bees; bumblebees visited *E. wildpretii* more frequently for nectar only (72.6% of all bumblebee visits) than for pollen, while the majority of the solitary bee visits were for pollen (86.3%). In total, 68.7% of all visits were for pollen in *E. wildpretii* (Table S4). In contrast, *Echium vulgare* (with high PA levels in the pollen) was visited by bees (76.5% of all visits) and Lepidoptera (23.5%). Among bees, bumblebee workers were the most abundant visitors (51.9% of the total visitors, 67.9% of the bees), followed by solitary bees (males and females together: 24.6% of all visitors, 32.1% of the bees). Bumblebee workers mostly visited *E. vulgare* for nectar only (73.1%), while solitary bees visited *E. vulgare* mostly for pollen (70.3%); and pollen-collecting bees constituted 31.3% of all visits (Table S4).

3.2 | Tests of the hypotheses

3.2.1 | Correlation between corolla and pollen PAs

In total, we detected 52 different PAs in the pollen of the species investigated (Table S2). Total PA concentrations in pollen of species of the *Echium*-type varied between 0.023 and 4.68 µg/mg (Table 1); between 0.003 and 4.98 µg/mg for the *Pulmonaria*-type; and between 0.0034 µg/mg and 0.91 for the *Borago*-type. PA concentrations in corollas ranged from 0.02 to 72.52 µg/mg (Table 1). PGLS analyses showed that there was a significant positive correlation between the levels of PAs in the pollen and those in the corolla (Figure 2; $F_{1,22}$ = 7,27, p = 0.013, λ = 0.837).

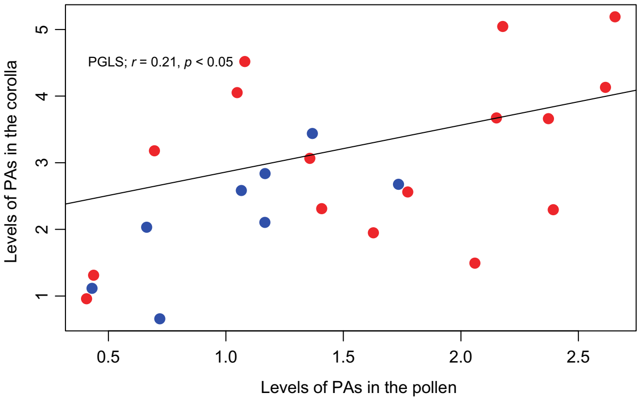

FIGURE 2 Relationship between the levels of PAs in the pollen and those in the corolla (fourth root transformed values in µg/mg) for 24 taxa of the Boraginaceae. *Borago*-type species in blue, *Echium*- and *Pulmonaria*-types in red

3.2.2 | Toxicity of pollen PAs to solitary bee larvae

The three main PAs (N-oxide and tertiary forms together) found in *H. adunca* provisions were echimidine (0.139 µg/mg, *SD* = 0.037), acetylvulgarine (0.018 µg/mg, *SD* = 0.03) and echivulgarine (0.361 µg/mg, *SD* = 0.031); the ratios of the N-oxide forms relative to the tertiary forms were 24.0%, 90.2% and 36.53%, respectively, for these three alkaloids (Table S5). Total PA values in the provisions (on average 0.561 µg/mg, *SD* = 0.055) were approximately six times lower than the concentrations measured in pure pollen, likely because of the high quantities of nectar found in natural provisions of *H. adunca* (Cane, Gardner, & Harrison, 2011; Westrich, 1989). Total PA levels in the control provisions were close to 0 (average 0.005 µg/mg, *SD* = 0.005), indicating that little pollen from PA-producing plants was included among the natural pollen hosts of the bees investigated. PA levels in PA-supplemented provisions (average across the three species investigated 0.399 µg/mg, *SD* = 0.116) were comparable to the levels found in the provisions of *H. adunca*; levels of PAs in the provisions remained stable over all experiments from the onset of larval development to cocoon spinning (Table S5).

Of 15 larvae of *C. rapunculi* developing on treatment diets, 11 were able to spin a cocoon, compared to all 15 larvae in the control group (Table 2). Larval survival from egg hatching to cocoon spinning was not significantly different between treatment and control (Kaplan–Meier statistics, χ^2 = 3.3, *df* = 1, p = 0.07). However, after cocoon spinning none of the larvae in the treatment successfully pupated (Table 2), while all 15 control individuals pupated and developed into adults (chi-squared test, χ^2 = 30.0, *df* = 1, p < 0.001). Of the 15 larvae of *O. bicornis* developing on the PA-treatment diets, 14 reached the cocoon stage versus 15 in the control diet (Kaplan–Meier statistics, χ^2 = 1, *df* = 1, p = 0.32). None of the larvae feeding

TABLE 2 Comparison of larval survival and survival to adult in three solitary bee species on control (natural provisions) and on provisions supplemented with amounts of pyrrolizidine alkaloids (PA) corresponding to those estimated in pure pollen and nectar provisions of *Echium vulgare*. Larval survival was tested with Kaplan–Meier statistics, the proportion of surviving adults with chi-squared tests (see text for details)

Bee species	Diet	Number of replicates	Surviving larvae	Kaplan–Meier probability	Surviving pupae	Surviving adults	Chi-squared probability
Chelostoma rapunculi	Control	15	15	0.06	15	15	<0.001
	PA-supplemented	15	11		0	0	
Osmia bicornis	Control	15	15	0.32	14	14	<0.001
	PA-supplemented	15	14		0	0	
Osmia cornuta	Control	15	15	NA	15	15	0.23
	PA-supplemented	15	15		15	13	

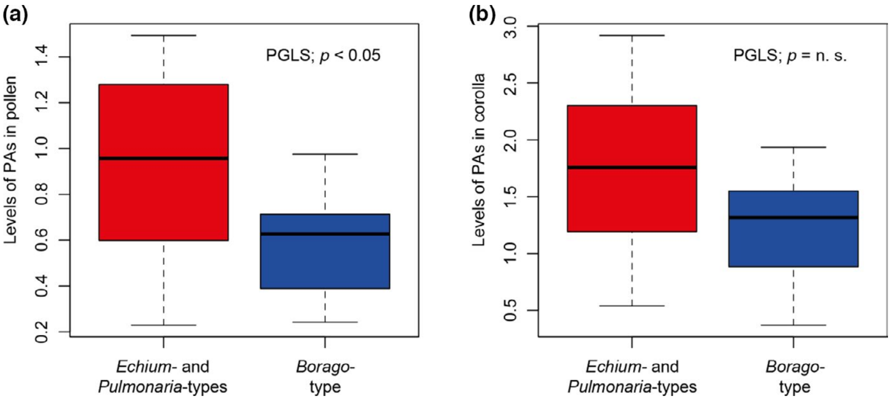

FIGURE 3 Boxplots of PA levels (fourth root transformed values in µg/mg) in the pollen (a) and in the corolla (b) of the Boraginaceae species investigated and separated into two groups: *Borago*-type species (blue), and *Pulmonaria*- and *Echium*-types (red)

on PA diet pupated, while 14 of 15 larvae pupated and developed into adults in the control diet (chi-squared test, $\chi^2 = 26.3$, $df = 1$, $p < 0.001$). In contrast, all 15 larvae of *O. cornuta* reached the pupal stage on PA-supplemented provisions, and of these 15 pupae, 13 produced adults (15 of 15 in the control; chi-squared test, $\chi^2 = 1.07$, $df = 1$, $p = 0.23$; Table 2). Adult weight was not significantly lower in the treatment than in the control although there was a non-significant trend (ANOVA; treatment effect: $F_{1,24} = 3.4$, $p = 0.08$; sex effect: $F_{1,24} = 213.4$, $p < 0.001$).

3.2.3 | Difference in pollen PA concentration between plant groups

Borago-type species had significantly lower PA levels in the pollen than other species (Figure 3a; PGLS statistics, $F_{1,24} = 4.89$, $p = 0.037$, $\lambda = 0$), but groups did not significantly differ for PA levels in the corolla (Figure 3b; PGLS statistics, $F_{1,22} = 1.176$, $p = 0.29$, $\lambda = 0.93$). Phylogenetic signal was low and not significantly different from zero for pollen PAs (kappa = 0.23, $p = 0.16$; Figure 4), while it was significantly different from zero for corolla (kappa = 0.53, $p = 0.002$; Figure 4). Since two values were missing for corollas, we repeated the test for pollen PAs under exclusion of these two species, resulting in a similar pattern: groups significantly differ in the levels of pollen PAs (PGLS statistics, $F_{1,22} = 4.38$, $p = 0.048$, $\lambda = 0$) and the phylogenetic signal was not significantly different from zero (kappa = 0.21, $p = 0.26$). The phylogenetic relationship among the species investigated is presented on Figure 4.

For pollen PAs, MVMORPH analyses strongly favoured an OU model with one optimum over a BM model (AIC values 49.68 and 31.98 for BM and OU respectively; Table S6); an OU model with two optima (OUM; one for species of the *Borago*-type and one for the other species) was favoured over an OU model with one optimum (AIC 29.80 for OUM; Table S6; likelihood ratio test, $p = 0.027$). Results were similar when the two species with missing corolla values were excluded (AIC 48.09, 30.36 and 28.80 for BM, OU and OUM respectively; likelihood ratio test for comparing OU and OUM, $p = 0.034$). In contrast, a BM model was favoured over both OU and OUM models for PA levels in the corollas (AIC 52.19, 57.20 and 52.76 for BM, OU and OUM respectively; Table S6).

3.2.4 | PA concentrations in pollen and floral specialization in bees

Boraginaceae taxa hosting at least one specialist bee (Table 1) did not have higher PA concentrations in the pollen than taxa not known to host specialist bees (PGLS statistics, $F_{1,15} = 0.05$ and 0.04, $p = 0.82$ and 0.84 for lambda values of 1.0 and 0.1 respectively). We found no significant relationship between the number of specialist bees and PA levels in the pollen (PGLS statistics: $F_{1,15} = 0.54$, $p = 0.48$; $\lambda = 0$).

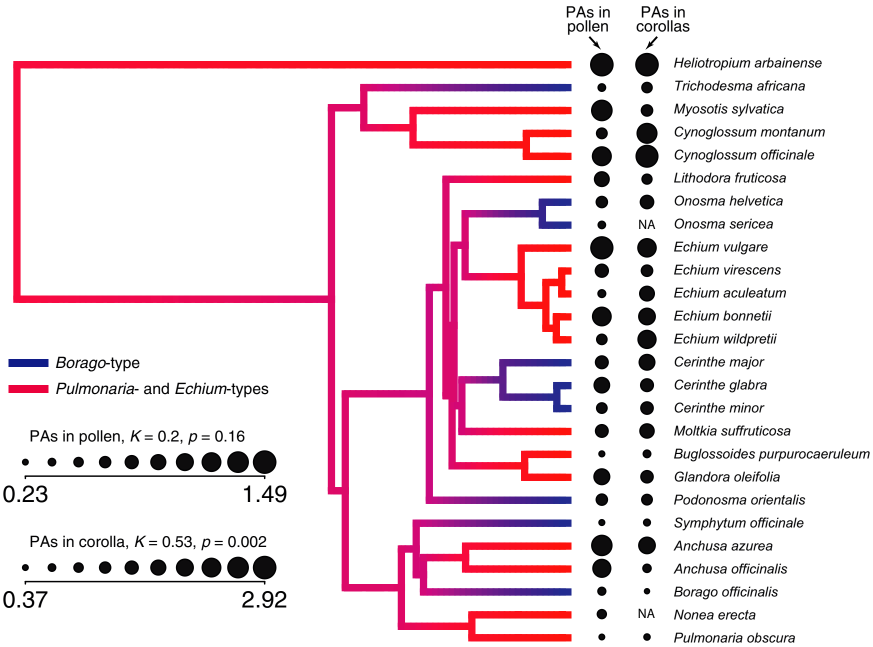

FIGURE 4 Phylogenetic tree showing the 26 species of Boraginaceae investigated; the size of the circles to the left of the species names is proportional to the levels of PAs in the pollen and in the corollas. Branches are coloured according to two groups: *Borago*-type species in blue, *Echium*- and *Pulmonaria*-types in red. *K* and *p* values refer to kappa statistics used to examine the phylogenetic signal of the PA levels (see text)

4 | DISCUSSION

Our study is the first to examine evolutionary trends of levels of pollen secondary compound concentration in a plant clade and to relate these levels to the plants' pollination biology. While the significant correlation between PA concentrations in the pollen and in the corolla (Figure 2) suggests that baseline levels of toxins in the pollen may be due to pleiotropy effects, our analyses of trait evolution suggest that pollen toxin levels are under stronger selection than levels in the corollas (Figures 3 and 4; Table S6). In contrast with previous hypotheses suggesting that selection increases toxin levels in the pollen to deter pollen robbers, we propose that in Boraginaceae selection by legitimate pollinators reduces baseline levels of toxins in the pollen of pollen-rewarding species, as has been suggested recently for the plant genus *Lupinus* (Heiling et al., 2019).

4.1 | Correlation between PA concentrations in the pollen and in the corolla

In agreement with our first prediction, levels of PAs in the corolla and in the pollen were significantly correlated in the examined species of Boraginaceae. This finding complements earlier studies demonstrating a correlation between the levels of toxins in nectar or pollen and those in other plant tissues, either among individuals in one species (Adler, Wink, Distl, & Lentz, 2006; Kessler & Halitschke, 2009) or in a plant clade using a phylogenetic framework (Adler, Seifert, Wink, & Morse, 2012; Manson et al., 2012). These four studies as well as our results suggest that the presence of toxic compounds in floral rewards may at least in part be physiologically constrained. As mentioned above, however, our evolutionary models clearly reject pure pleiotropy effects; in addition the correlation, albeit significant, accounted for only 21% of total variance, implying that other evolutionary forces also act on PA levels in the pollen.

4.2 | Effects of PAs on pre-imaginal development

While the impact of nectar toxins on pollinators has received much attention, much less is known on the impact of pollen toxins, especially on solitary bees. Previous egg-transfer experiments demonstrated that larvae of the solitary bees *Chelostoma rapunculi* and *Osmia bicornis* could not develop on pure pollen and nectar provisions of *Echium vulgare*, in contrast to larvae of *O. cornuta* (Praz et al., 2008a; Sedivy et al., 2011). In our bioassays, larvae of the same three species were reared on their host pollen provisions supplemented with the amounts of PAs measured in pure provisions of *E. vulgare*. In agreement with these previous studies, no adult was produced in *C. rapunculi* and *O. bicornis*, while 13 of 15 individuals successfully reached the adult stage in *O. cornuta*. These bioassays thus verify our second hypothesis and demonstrate for the first time that pollen secondary compounds can strongly impact the pre-imaginal development of solitary bees, adding to previous evidence gathered in eusocial bees (Arnold et al., 2014; Lucchetti et al., 2018). In our experiments, mortality occurred comparatively late in development, during or just before the pupal stage. As a result, larval survival (as opposed to survival to adult) was not significantly different from the controls, in contrast to previous studies (Praz et al., 2008a; Sedivy et al., 2011). In our bioassays, bee larvae were developing on pollen and nectar provisions from their respective host plants and not on non-host *Echium* provisions as in previous studies. Since multiple factors likely influence the suitability of pollen provisions for bees (Praz et al., 2008a; Vanderplanck et al., 2018), the impact of toxic compounds is expected to be stronger on non-host pollen diets than on host-pollen diets. In agreement, *O. bicornis* larvae were shown to suffer high mortality on *Borago* pollen (Bukovinszky et al., 2017), although according to our study this pollen type only exhibits trace amounts of PAs. To summarize, we observed that larval provisions supplemented with realistic levels of pollen toxins had a negative impact on the pre-imaginal development of non-specialized bee species, supporting the idea that these compounds can strongly impact the fitness of pollen-feeding visitors.

4.3 | Levels of PAs in the pollen

In agreement with our third prediction, species of the *Borago*-type, which are nearly exclusively pollinated by bees and reward them with both pollen and nectar, exhibited significantly lower levels of PAs in the pollen than the other species, which are in most cases pollinated by nectar foragers. Several evolutionary processes may account for these patterns in Boraginaceae. In species of the *Pulmonaria*-type, we hypothesize that baseline levels of PAs in pollen are present due to physiological spillover from other floral tissues, and not primarily because of interactions with pollinators or bees. Consistent with this hypothesis, the highest levels of PAs measured in our study, both in pollen and corollas, were found in *Heliotropium*. In one species of this plant genus, *H. indicum*, 70% of all PAs contained in the plant were found in the inflorescence (El-Shazly & Wink, 2014) in contrast to most other species of Boraginaceae, in which high PA levels are found in leaves or in roots. The high levels of PAs in the pollen of *Heliotropium* are unlikely driven by bee–flower interactions, since in this genus the pollen is concealed in a particularly narrow floral tube and only very few specialized bee species equipped with modified mouthparts can harvest it (Gotlieb et al., 2014). Based on our field observations, these bees represent a small fraction of the numerous nectar-visiting insects and their impact on the reproduction of the plant (either as pollinators or pollen robbers) is most likely limited. However, one fact that contradicts the pleiotropy hypothesis in *Heliotropium* is that several PAs found in the pollen were not found in the corolla (Table S2). Further examination of the qualitative differences in PAs across floral tissues, and not only of the quantitative differences as done in our study, is needed to further test the pleiotropy hypothesis. In addition, in *Myosotis sylvatica* (as well as in *Anchusa officinalis* and, to some extent, in *Borago officinalis*) the higher levels of PAs in the pollen than in the corollas (Table 1) contradict pure pleiotropy effects. The floral morphology of *Myosotis* is similar to that of *Heliotropium*, with a particularly narrow floral tube concealing the anthers, and this plant genus has never been reported to be visited by pollen-collecting insects (Table S1). Future research should examine whether specific pollen PAs have an antimicrobial function in these genera, or whether they originate from spillover from other floral tissues not investigated here. These instances, especially the cases of *Myosotis* and *Anchusa officinalis* where toxin levels are considerably higher in the pollen than in the corolla, suggest that selection may also act to increase PA concentrations in pollen relative to baseline levels.

Not all members of the *Pulmonaria*-type exhibit high levels of PAs in the pollen. Several genera, such as *Pulmonaria*, *Glandora*, *Lithodora* and *Buglossoides*, had PA levels in pollen as low as species of the *Borago*-type. Interestingly, these genera, although visited by a vast array of nectar-foraging insects, host at times large populations of anthophorine bees (Ferrero et al., 2011). Anthophorines often have modified mouthparts which allow them to extract the pollen from narrow corolla tubes such as those observed in these genera. While pollination has been suggested to occur predominantly during nectar visits of both bees and other insects (Ferrero et al., 2011), it may still be an advantage for these plants to support large populations of bees by rewarding them with both toxin-free pollen and nectar. Under this scenario, selection may act to lower baseline levels of secondary compounds in floral rewards of these plant species (Heiling et al., 2019).

Similarly, in species of the *Borago*-type, the low PA levels detected are in agreement with their exclusive dependency on bees. We postulate that toxic compounds in the pollen of *Borago*-type species would lead to decreased visitation rates of their exclusive pollinators, and consequently that selection has lowered their levels in pollen (Figure 3a) and possibly indirectly also in the corolla (Figure 3b). These toxic compounds in the pollen (or in floral tissues such as corolla) may impact bee visitation rates, and thus pollen transfer and plant reproduction success, in two ways. First, they may deter pollen-collecting bees because of altered floral volatile cues (Kessler & Halitschke, 2009) or pollen taste (Muth, Francis, & Leonard, 2016). Second, pollen toxins may impact larval survival and reduce bee reproductive success when incorporated into larval diets. These two mechanisms may shape pollen host selection by bees at different time-scales. Sensitivity to fine changes in floral cues or pollen taste may modulate short-term choices by generalist bees, such as bumblebees, and guide them in selecting among different possible host plant species and among plant individuals within the same species (Wang et al., 2019). At a longer time-scale, the presence of toxic compounds in the pollen may impact the innate floral preferences of more specialized bees in order to minimize larval exposure to toxins by triggering and maintaining associations with host plants offering suitable pollen. This second mechanism would be especially true for solitary bees, many of which show innate preferences for a restricted spectrum of host plants (Praz, Müller, & Dorn, 2008b).

In *Echium*, we found higher PA levels in the pollen of *E. vulgare*, which was predominantly visited by nectar-foraging visitors in our survey, than in that of *E. wildpretii*, which was mostly visited by pollen-collecting bees (Table S4). In contrast, PA levels in the corolla were comparable in these two plant species (Table 1). Although these results are tentative and based on limited observations on just two species, we suggest that different pollinator communities in the different habitats of these *Echium* species have selected for different levels of PAs in the pollen. In the temperate habitats of *E. vulgare*, bumblebees are abundant, and the bloom of *E. vulgare* in early summer corresponds to a time of year where bumblebee colonies produce large numbers of workers. The ratio of nectar-to-pollen consumption is twice as high for bumblebees than for solitary bees

(Cane et al., 2011). Consequently, the deterrent effect of pollen PAs may not compromise pollination given the large pool of nectar foragers. Moreover, if pollen-collecting bumblebees were less efficient pollinators than nectar foragers, for example because they groom the pollen off their body between visits (Thomson, 1986), selection may even maintain or increase pollen levels of PAs to deter pollen collection (Wang et al., 2019). Pollinator communities are very different in the arid Mediterranean habitats of *E. wildpretii* on the Canary Islands, with fewer bumblebees and more solitary bees compared to habitats in temperate Europe. Different pollinator assemblages may have driven the need for *E. wildpretii* to mediate relationships with pollen-collecting solitary bees by offering both nectar and suitable pollen as rewards, and thus as in *Borago*-type species, selection may have reduced the levels of toxic metabolites in pollen. Future research is needed to elucidate the impact of PAs in the pollen of members of the diverse genus *Echium*, for example by experimentally comparing the behaviour, reproductive success and pollination efficiency of different pollinators visiting *Echium* species differing in PA levels in the pollen.

4.4 | PA level and specialization in bees

In contrast to our fourth prediction, we found no indication that solitary bees specialize on plants that exhibit particularly high levels of PAs in the pollen. Bees specialize on all three groups of Boraginaceae, with no preference for species of the *Pulmonaria* or *Echium*-types, which exhibit comparatively high PA levels, over those of the *Borago*-type. Two genera hosting numerous bee specialists had comparatively high levels of PAs in the pollen (*Heliotropium*, *Echium*); interestingly in both cases nearly all specialist bees belong to two lineages (the genus *Haetosmia* on *Heliotropium* and the osmiine subgenus *Hoplitis* on *Echium*), suggesting that diversification has occurred in these bee clades after the shift to these host plants took place. However, other plant genera with similarly high levels do not host specialists, for example the widespread plant genus *Myosotis*, with comparable floral morphology to *Heliotropium*. We hypothesize that bees have a tendency to specialize on Boraginaceae hosts that represent predictable and abundant resources in regions where bee faunas are particularly numerous and diverse (Minckley, Cane, & Kervin, 2000). Boraginaceae taxa that host more than three species of specialist bees (the genera *Onosma*, *Heliotropium* and *Echium*) all reach their maximal diversity and abundance in Mediterranean (*Onosma* and *Echium*) or xeric regions (Palaearctic species of *Heliotropium*). Consequently, radiation of these plant taxa has likely taken place in regions hosting diverse bee faunas with high incidence of oligolecty (Danforth et al., 2019). Boraginaceae genera such as *Cerinthe*, *Myosotis*, *Pulmonaria* and *Symphytum* have their centres of diversity in temperate or mountainous regions, which host less diverse bee faunas with a higher proportion of floral generalists, such as bumblebees. That there is no direct link between the presence of toxin in the pollen and specialization by bees is further substantiated by the fact that several bee species specialized onto *Anchusa* visit *A. officinalis* for pollen, but not *A. azurea*, which exhibits higher PA levels than *A. officinalis*.

5 | CONCLUSIONS

Our results suggest that Boraginaceae primarily protect their pollen against pollen robbers using complex floral structures and not through secondary chemistry. We propose that the presence of high levels of toxic compounds in pollen is in most cases not linked to plant–pollinator interactions in Boraginaceae. Rather, baseline levels of pollen toxins may at least in part be due to physiological spillover from other floral tissues. The pattern observed suggests that selection has led to decreased levels of PAs in the pollen of plant taxa relying on pollen-collecting bees for their pollination (Heiling et al., 2019). These hypotheses, together with the demonstration of the potentially negative effects of pollen PAs on pollinator fitness, contribute to the view that toxins in floral rewards can represent a detrimental side effect of the plant's defence chemistry against herbivores or other antagonists, and thus bear ecological costs (Jones & Agrawal, 2016; Kessler & Halitschke, 2009).

ACKNOWLEDGEMENTS

We thank Claudio Sedivy for providing nests of solitary bees, Laurent Oppliger and the Botanical Garden of Neuchatel for assistance with bee rearings, Maryse Vanderplanck for help in the field, Damien Thevenet for help in PAs isolation. We also thank Julien Vieu, Anne-Marie Labouche and Romain Piault for help with statistical analyses and Jessica Litman for English correction and numerous comments on previous versions of the Manuscript. This study was funded by the University of Neuchâtel and the Foundation Dr. Joachim de Giacomi; M.L.'s salary was supported by the Swiss Bee Research Centre of Agroscope. Detailed comments by the subject editor and by two anonymous reviewers substantially improved the manuscript.

AUTHORS' CONTRIBUTIONS

C.J.P. and G.G. designed research; V.T., M.A.L., C.K., G.G. and C.J.P. conducted the experiments and chemical analyses; V.T., M.A.L., D.B., C.K., G.A.D., A.D. and C.J.P. provided samples and performed field observations; C.J.P., D.B. and S.R. performed statistical analyses; and C.J.P. and S.R. wrote the paper with contributions from all authors.

DATA AVAILABILITY STATEMENT

Data deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.qbzkh18f4 (Praz et al., 2020).

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REFERENCES

Adler, L. S., Seifert, M. G., Wink, M., & Morse, G. E. (2012). Reliance on pollinators predicts defensive chemistry across tobacco species. *Ecology Letters*, *10*, 1140–1148. https://doi.org/10.1111/j.1461-0248.2012.01838.x

Adler, L. S., Wink, M., Distl, M., & Lentz, A. J. (2006). Leaf herbivory and nutrients increase nectar alkaloids. *Ecology Letters*, *9*, 960–967. https://doi.org/10.1111/j.1461-0248.2006.00944.x

Aloisi, I., Cai, G., Serafini-Fracassini, D., & Del Duca, S. (2016). Polyamines in pollen: From microsporogenesis to fertilization. *Frontiers in Plant Science*, *7*, 155. https://doi.org/10.3389/fpls.2016.00155

Arnold, S. E. J., Idrovo, M. E. P., Arias, L. J. L., Belmain, S. R., & Stevenson, P. C. (2014). Herbivore defence compounds occur in pollen and reduce bumblebee colony fitness. *Journal of Chemical Ecology*, *40*, 878–881. https://doi.org/10.1007/s10886-014-0467-4

Bukovinszky, T., Rikken, I., Evers, S., Wäckers, F. L., Biesmeijer, J. C., Prins, H. H. T., & Kleijn, D. (2017). Effects of pollen species composition on the foraging behaviour and offspring performance of the mason bee *Osmia bicornis* (L.). *Basic and Applied Ecology*, *18*, 21–30. https://doi.org/10.1016/j.baae.2016.11.001

Butler, M., & King, A. (2004). Phylogenetic comparative analysis: A modeling approach for adaptive evolution. *The American Naturalist*, *164*, 683–695. https://doi.org/10.1086/426002

Cane, J., Gardner, D. R., & Harrison, P. A. (2011). Nectar and pollen sugars constituting larval provisions of the alfalfa leaf-cutting bee (*Megachile rotundata*) (Hymenoptera: Apiformes: Megachilidae). *Apidologie*, *42*, 401–408. https://doi.org/10.1007/s13592-011-0005-0

Clavel, J., Escarguel, G., & Merceron, G. (2015). mvMORPH: An R package for fitting multivariate evolutionary models to morphometric data. *Methods in Ecology and Evolution*, *6*, 1311–1319. https://doi.org/10.1111/2041-210X.12420

Cohen, J. I. (2014). A phylogenetic analysis of morphological and molecular characters of Boraginaceae: Evolutionary relationships, taxonomy, and patterns of character evolution. *Cladistics*, *30*, 139–169. https://doi.org/10.1111/cla.12036

Cook, D., Manson, J. S., Gardner, D. R., Welch, K. D., & Irwin, R. E. (2013). Norditerpene alkaloid concentrations in tissues and floral rewards of larkspurs and impacts on pollinators. *Biochemical Systematics and Ecology*, *48*, 123–131. https://doi.org/10.1016/j.bse.2012.11.015

Corbet, S. A. (1978). Bee visits and the nectar of *Echium vulgare* L. and *Sinapis alba* L. *Ecological Entomology*, *3*, 25–37. https://doi.org/10.1111/j.1365-2311.1978.tb00900.x

Danforth, B. N., Minckley, R. L., & Neff, J. L. (2019). *The solitary bees. Biology, evolution, conservation*. Princeton, NJ: Princeton University Press.

El-Shazly, A., & Wink, M. (2014). Diversity of pyrrolizidine alkaloids in the Boraginaceae – Structures, distribution, and biological properties. *Diversity*, *6*, 188–282. https://doi.org/10.3390/d6020188

Faegri, K. (1986). The solanoid flower. *Transactions of the Botanical Society of Edinburgh*, *45*, 51–59. https://doi.org/10.1080/03746608608684993

Ferrero, V., Castro, S., Sánchez, J. M., & Navarro, L. (2011). Stigma–anther reciprocity, pollinators, and pollen transfer efficiency in populations of heterostylous species of *Lithodora* and *Glandora* (Boraginaceae). *Plant Systematics and Evolution*, *291*, 267–276. https://doi.org/10.1007/s00606-010-0387-x

Gosselin, M., Michez, D., Vanderplanck, M., Roelants, D., Glauser, G., & Rasmont, P. (2013). Does *Aconitum septentrionale* chemically protect floral rewards to the advantage of specialist bumblebees? *Ecological Entomology*, *38*, 400–407. https://doi.org/10.1111/een.12032

Gotlieb, A., Pisanty, G., Rozen, J. G., Müller, A., Röder, G., Sedivy, C., & Praz, C. (2014). Nests, floral preferences, and immatures of the bee *Haetosmia vechti* (Hymenoptera: Megachilidae: Osmiini). *American Museum Novitates*, *3808*, 1–20. https://doi.org/10.1206/3808.1

Goulson, D. (2003). *Bumblebees, their behaviour and ecology*. Oxford, UK: Oxford University Press.

Haider, M., Dorn, S., & Müller, A. (2013). Intra- and interpopulational variation in the ability of a solitary bee species to develop on non-host pollen: Implications for host range expansion. *Functional Ecology*, *27*, 255–263. https://doi.org/10.1111/1365-2435.12021

Haider, M., Dorn, S., & Müller, A. (2014). Better safe than sorry? A Fabaceae species exhibits unfavourable pollen properties for developing bee larvae despite its hidden anthers. *Arthropod-Plant Interactions*, *8*, 221–231. https://doi.org/10.1007/s11829-014-9299-y

Hartmann, T. (1999). Chemical ecology of pyrrolizidine alkaloids. *Planta*, *207*, 483–495. https://doi.org/10.1007/s004250050508

Heiling, J. M., Cook, D., Lee, S. T., & Irwin, R. E. (2019). Pollen and vegetative secondary chemistry of three pollen-rewarding lupines. *American Journal of Botany*, *106*, 643–655. https://doi.org/10.1002/ajb2.1283

Irwin, R. E., Cook, D., Richardson, L., Manson, J., & Gardner, D. (2014). Secondary compounds in floral rewards of toxic rangeland plants: Impacts on pollinators. *Journal of Agricultural and Food Chemistry*, *62*, 7335–7344. https://doi.org/10.1021/jf500521w

Jones, P. L., & Agrawal, A. A. (2016). Consequences of toxic secondary compounds in nectar for mutualist bees and antagonist butterflies. *Ecology*, *97*, 2570–2579. https://doi.org/10.1002/ecy.1483

Kessler, A., & Halitschke, R. (2009). Testing the potential for conflicting selection on floral chemical traits by pollinators and herbivores: Predictions and case study. *Functional Ecology*, *23*, 901–912. https://doi.org/10.1111/j.1365-2435.2009.01639.x

LoCascio, G. M., Aguirre, L., Irwin, R. E., & Adler, L. S. (2019). Pollen from multiple sunflower cultivars and species reduces a common bumblebee gut pathogen. *Royal Society Open Science*, *6*, 190279. https://doi.org/10.1098/rsos.190279

Lucchetti, M. A., Glauser, G., Kilchenmann, V., Dübecke, A., Beckh, G., Praz, C., & Kast, C. (2016). Pyrrolizidine alkaloids from *Echium vulgare* in honey originate primarily from floral nectar. *Journal of Agricultural and Food Chemistry*, *64*, 5267–5273. https://doi.org/10.1021/acs.jafc.6b02320

Lucchetti, M. A., Kilchenmann, V., Glauser, G., Praz, C., & Kast, C. (2018). Nursing protects honeybee larvae from secondary metabolites of pollen. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1875), 20172849. https://doi.org/10.1098/rspb.2017.2849

Macel, M. (2011). Attract and deter: A dual role for pyrrolizidine alkaloids in plant–insect interactions. *Phytochemistry Reviews*, *10*, 75–82. https://doi.org/10.1007/s11101-010-9181-1

Manson, J. S., Rasmann, S., Halitschke, R., Thomson, J. D., & Agrawal, A. A. (2012). Cardenolides in nectar may be more than a consequence of allocation to other plant parts: A phylogenetic study of *Asclepias*. *Functional Ecology*, *26*, 1100–1110. https://doi.org/10.1111/j.1365-2435.2012.02039.x

Minckley, R. L., Cane, J. H., & Kervin, L. (2000). Origins and ecological consequences of pollen specialization among desert bees. *Proceedings of the Royal Society B*, *267*, 265–271. https://doi.org/10.1098/rspb.2000.0996

Müller, A. (1995). Morphological specializations in central European bees for the uptake of pollen from flowers with anthers hidden in narrow corolla tubes (Hymenoptera: Apoidea). *Entomologia Generalis*, *20*, 43–57. https://doi.org/10.1127/entom.gen/20/1995/43

Müller, A. (2019). *Palaearctic osmiine bees*. Retrieved from http://blogs.ethz.ch/osmiini

Muth, F., Francis, J. S., & Leonard, A. S. (2016). Bees use the taste of pollen to determine which flowers to visit. *Biology Letters*, *12*, 20160356. https://doi.org/10.1098/rsbl.2016.0356

Orme, D., Freckleton, R., Thomas, G., Fritz, S., Isaac, N., & Pearse, W. (2013). *Caper: Comparative analyses of phylogenetics and evolution in R*. R package version 1.0.1. Retrieved from https://CRAN.R–project.org/package=caper

Palmer-Young, E. C., Farrell, I. W., Adler, L. S., Milano, N. J., Egan, P. A., Junker, R. R., … Stevenson, P. C. (2019). Chemistry of floral rewards: Intra- and interspecific variability of nectar and pollen secondary metabolites across taxa. *Ecological Monographs*, *89*, e01335. https://doi.org/10.1002/ecm.1335

Praz, C. J., Müller, A., & Dorn, S. (2008a). Specialized bees fail to develop on non-host pollen: Do plants chemically protect their pollen? *Ecology*, *89*, 795–804. https://doi.org/10.1890/07-0751.1

Praz, C. J., Müller, A., & Dorn, S. (2008b). Host recognition in a pollen-specialist bee: Evidence for a genetic basis. *Apidologie*, *39*, 547–557. https://doi.org/10.1051/apido:2008034

Praz, C., Trunz, V., Lucchetti, M., Bénon, D., Dorchin, A., Desurmont, G., … Glauser, G. (2020). Data from: To bee or not to bee: The "raison d'être" of toxic secondary compounds in the pollen of Boraginaceae. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.qbzkh18f4

R Core Team. (2017). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from www.r-project.org

Revell, L. J. (2012). phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, *3*, 217–223. https://doi.org/10.1111/j.2041-210X.2011.00169.x

Rivest, S., & Forrest, J. R. K. (2020). Defence compounds in pollen: Why do they occur and how do they affect the ecology and evolution of bees. *New Phytologist*, *225*, 1053–1064. https://doi.org/10.1111/nph.16230

Sedivy, C., Dorn, S., Widmer, A., & Müller, A. (2013). Host range evolution in a selected group of osmiine bees (Hymenoptera: Megachilidae): The Boraginaceae-Fabaceae paradox. *Biological Journal of the Linnean Society*, *108*, 35–54. https://doi.org/10.1111/j.1095-8312.2012.02013.x

Sedivy, C., Müller, A., & Dorn, S. (2011). Closely related pollen generalist bees differ in their ability to develop on the same pollen diet: Evidence for physiological adaptations to digest pollen. *Functional Ecology*, *25*, 718–725. https://doi.org/10.1111/j.1365-2435.2010.01828.x

Sedivy, C., Piskorski, R., Müller, A., & Dorn, S. (2012). Too low to kill: Concentration of the secondary metabolite ranunculin in buttercup pollen does not affect bee larval survival. *Journal of Chemical Ecology*, *38*, 996–1002. https://doi.org/10.1007/s10886-012-0153-3

Sedivy, C., Praz, C. J., Müller, A., Widmer, A., & Dorn, S. (2008). Patterns of host-plant choice in bees of the genus *Chelostoma*: The constraint hypothesis of host-range evolution in bees. *Evolution*, *62*, 2487–2507. https://doi.org/10.1111/j.1558-5646.2008.00465.x

Somerville, D. C., & Nicol, H. I. (2006). Crude protein and amino acid composition of honey bee-collected pollen pellets from south-east Australia and a note on laboratory disparity. *Australian Journal of Experimental Agriculture*, *46*, 141–149. https://doi.org/10.1071/EA03188

Spear, D. M., Silverman, S., & Forrest, J. R. K. (2016). Asteraceae pollen provisions protect *Osmia* mason bees (Hymenoptera: Megachilidae) from brood parasitism. *The American Naturalist*, *187*, 797–803. https://doi.org/10.1086/686241

Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, *30*, 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Stevenson, P. C., Nicolson, S. W., & Wright, G. A. (2017). Plant secondary metabolites in nectar: Impacts on pollinators and ecological functions. *Functional Ecology*, *31*, 65–75. https://doi.org/10.1111/1365-2435.12761

Taylor, L. P., & Grotewold, E. (2005). Flavonoids as developmental regulators. *Current Opinion in Plant Biology*, *8*, 317–323. https://doi.org/10.1016/j.pbi.2005.03.005

Teppner, H. (2011). Flowers of Boraginaceae (*Symphytum*, *Onosma*, *Cerinthe*) and *Andrena symphyti* (Hymenoptera-Andrenidae): Morphology, pollen portioning, vibratory pollen collection, nectar robbing. *Phyton*, *50*, 145–180.

Therneau, T. M., & Lumley, T. (2019). *Package 'survival' in R*. R package version 2.44-1.1. Retrieved from https://cran.r–project.org/web/packages/survival

Thomson, J. D. (1986). Pollen transport and deposition by bumble bees in *Erythronium*: Influences of floral nectar and bee grooming. *Journal of Ecology*, *74*, 329–341. https://doi.org/10.2307/2260258

Vanderplanck, M., Decleves, S., Roger, N., Decroo, C., Caulier, G., Glauser, G., … Michez, D. (2018). Is non-host pollen suitable for generalist bumblebees? *Insect Science*, *25*, 259–272. https://doi.org/10.1111/1744-7917.12410

Vogel, S. (1996). Christian Konrad Sprengel's theory of the flower: The cradle of floral ecology. In D. G. Lloyd & S. C. H. Barrett (Eds.), *Floral biology* (pp. 44–66). Boston, MA: Springer.

Wang, X., Tang, J., Wu, T., Wu, D., & Huang, S. (2019). Bumblebee rejection of toxic pollen facilitates pollen transfer. *Current Biology*, *29*, 1–6. https://doi.org/10.1016/j.cub.2019.03.023

Westrich, P. (1989). *Die Wildbienen Baden-Württembergs*. Stuttgart, Germany: Ulmer.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Trunz V, Lucchetti MA, Bénon D, et al. To bee or not to bee: The 'raison d'être' of toxic secondary compounds in the pollen of Boraginaceae. *Funct Ecol*. 2020;34:1345–1357. https://doi.org/10.1111/1365-2435.13581