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The evolution of signal–reward correlations in bee- and hummingbird-pollinated species of *Salvia*

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Within-individual variation in floral advertising and reward traits is a feature experienced by pollinators that visit different flowers of the same plant. Pollinators can use advertising traits to gather information about the quality and amount of rewards, leading to the evolution of signal–reward correlations. As long as plants differ in the reliability of their signals and pollinators base their foraging decisions on this information, natural selection should act on within-individual correlations between signals and rewards. Because birds and bees differ in their cognitive capabilities, and use different floral traits as signals, we tested the occurrence of adaptive divergence of the within-individual signal–reward correlations among *Salvia* species that are pollinated either by bees or by hummingbirds. They are expected to use different floral advertising traits: frontal traits in the case of bees and side traits in the case of hummingbirds. We confirmed this expectation as bee- and hummingbird-pollinated species differed in which specific traits are predominantly associated with nectar reward at the within-individual level. Our findings highlight the adaptive value of within-individual variation and covariation patterns, commonly disregarded as 'environmental noise', and are consistent with the hypothesis that pollinator-mediated selection affects the correlation pattern among floral traits.

1. Introduction

The functional value of advertising and reward traits expressed by flowers has historically been the focus of scientific research. Both advertisement traits and the amount and quality of floral rewards show significant levels of within-individual variation [1–7]. Although part of this variation in rewards may result from previous visits by pollinators, recent evidence indicates that a large portion of within-individual variance in floral traits is constitutive of the plant phenotype [5–7]. Hence, multi-flowering species are expected to express a complex trait resulting from the simultaneous variation of advertisement and reward traits. Although natural selection has been predicted to favour the association between advertising and reward traits [8–10], there have been few attempts to experimentally demonstrate the functional value of trait combinations [11]. Whereas a number of studies have addressed the multivariate structure of functional floral traits within populations [12–14], the functional value of the within-individual covariation in advertising and reward traits has rarely been studied. Thus, the expected within-individual relationship between floral signals and reward is still an untested assumption.

When a pollinator visits a sequence of flowers from the same plant, it experiences a continuum of variation in advertising traits and in the amount and quality of rewards (ranging from full rewarding to deceptive flowers [4]). Pollinators, in turn, are able to gather information about signal–reward correlation through associative learning [15,16], and they are able to combine this information with

Figure 1. Aspects of *Salvia* flowers. (*a*) Flowers of *S. fulgens*, a hummingbird-pollinated species. (*b*) Flowers of *S. pallida*, a bee-pollinated species. (*c*) Lateral and frontal view of an idealized *Salvia* flower. Measured areas in grey: (i) the lateral area of the corolla tube and (ii) the frontal area of the corolla lower lip. (Online version in colour.)

previous knowledge to assess patch quality [17]. As long as plants differ in the reliability of their signals and the pollinators base their foraging decisions on this information, natural selection is expected to act on within-individual correlation between signals and rewards. Accordingly, recent evidence has demonstrated that pollinator-mediated selection favours high levels of signal accuracy, increasing floral honesty in *Turnera ulmifolia* [18]. In addition, theoretical models have suggested that in repeated consumer–resource interactions, the ability of the consumer to verify the accuracy of the signal after it has responded to it (i.e. signal verification) can promote and maintain reliable signalling [19]. However, no studies are available assessing the evolution of signal–reward within-individual correlations using a macroevolutionary approach.

Given that pollinator species vary in their cognitive capabilities, foraging patterns and energetic requirements, they use different signal–reward combinations. For example, pollinators that must land on the flowers to forage (e.g. bees, flies and wasps) use mainly frontal advertisement floral traits, whereas pollinators that are able to hover and easily change their flight direction (e.g. hummingbirds, bats and hawkmoths) are expected to use side advertisement floral traits [20]. Thus, depending on their foraging abilities, pollinators should target different advertisement traits and hence their correlation with rewarding traits. An ideal system to test this hypothesis is the clade of Neotropical *Salvia* species, which has distinct bee and hummingbird pollination syndromes. Bee pollination is ancestral in this group, and evolution towards ornithophily includes modifications in length, colour and shape of the corolla, nectar guides, nectar quantity and composition, and in the lever mechanism of anthers [21]. During the evolutionary transition from bee to hummingbird pollination, the signal–reward correlations should have simultaneously experienced adaptive changes. In this study, we examined whether the evolution of ornithophily in *Salvia* subgenus *Calosphace* involved a change from frontally based to lateral advertising traits. We predicted greater within-individual covariation between the lateral area of the corolla and the amount of reward in hummingbird-pollinated species. By contrast, in bee-pollinated species, within-individual covariation between frontal advertising traits and rewards should have been selected.

Using comparative phylogenetic analyses, we tested the hypothesis of adaptive divergence of the signal–reward correlation between pollination syndromes. Following Hansen [22] and Butler & King [23], we discriminated among alternative neutral and adaptive hypotheses concerning the evolution of this quantitative trait. While neutral models assume a Brownian motion (BM) process of trait evolution, adaptive hypotheses use an Ornstein–Uhlenbeck (OU) process to model the evolution of one or more continuous traits. Each OU hypothesis predicts a different distribution of adaptive optima on the branches of a phylogeny corresponding to selection regimes that currently and historically operate on the study system. To this end, we estimated within-individual signal–reward correlation in a group of *Salvia* species including bee- and hummingbird-pollinated species, and built a molecular phylogeny as the raw material for the construction of different adaptive scenarios. If bird- and bee-pollinated species of *Salvia* evolved different patterns of signal–reward correlation, we predict that an OU hypothesis corresponding to two adaptive optima should better explain the variation in the signal–reward correlation among species than alternative adaptive or neutral explanations.

2. Material and methods

(a) Study species

Salvia subgenus *Calosphace* is a monophyletic group of Neotropical plants [24] distributed from the United States to Chile and Argentina. Their flowers present complex bilabiate or tubular floral architectures and contrasting pollination syndromes, with most of them pollinated by bees, and about one-third pollinated by hummingbirds [21]. We sampled 18 *Salvia* species from natural populations in Mexico and Argentina (figure 1*a*,*b*; electronic supplementary material, table S1): *S. atrocyanea*, *S. calolophos*, *S. cinnabarina*, *S. cuspidata* ssp. *gilliesii*, *S. elegans*, *S. fulgens*, *S. guaranitica*, *S. iodantha*, *S. lavanduloides*, *S. longispicata*, *S. mexicana*, *S. misella*, *S. pallida*, *S. personata*, *S. polystachya*, *S. purpurea*, *S. stachydifolia* and *S. thyrsiflora*. The main type of pollinator of each species was determined from literature [21,25,26] and confirmed with field observations for three of them (electronic supplementary material, table S2). Eight species (*S. atrocyanea*, *S. cinnabarina*, *S. elegans*, *S. fulgens*, *S. guaranitica*, *S. iodantha*, *S. mexicana* and *S. purpurea*) were mainly pollinated by hummingbirds, while the rest of the species were pollinated by bees.

Salvia mexicana and *S. purpurea* are highly variable species [21], and can be pollinated by both bees and hummingbirds. Our field observations, however, confirmed that hummingbirds were the main pollinators of *S. mexicana* at our study site (electronic supplementary material, table S2). In the case of *S. purpurea*, the flowers from our study site presented long corolla tubes and comparatively short and flexible lower lips, features that allow birds to access the nectar, excluding bees [21].

(b) Measured traits

We sampled 14–56 individuals per species and 3–15 flowers per individual (electronic supplementary material, table S1). For nectar measurements, plants were bagged at noon, and nectar volume and sugar concentration were recorded in the following day in freshly opened flowers using 1 or 5 µl micro-caps and

temperature-compensated hand refractometers (American Optical 10431). These variables were used to calculate nectar sugar content. Because flowers can accumulate nectar during the measuring period, we recorded the time at which each measurement was taken, and used the residuals from the regression between sugar content and time in the subsequent analyses. We took photographs of the side and front view of the same flowers used for nectar measurements along with a reference scale, using a Nikon D50 digital camera. Smaller flowers were photographed from closer distances, to ensure that flowers of different sizes occupied approximately the same relative area in the resulting photographs. For each photograph, we used the reference scale to transform the area measurements from pixels to square millimetres, following the instructions of UTHSCSA Image Tool v. 3.00 software. For each flower, we measured the frontal area of the lower lip and the lateral area of the corolla tube (figure 1*c*).

(c) Signal–reward correlation

When a research question involves assessing the joint change in the expression of two or more phenotypic traits, the sources of covariances must be distinguished. Because we were interested in the within-individual covariation of traits, we wanted to disentangle the within- and among-individual sources of covariation. Within-individual covariances are expected to be targets of pollinator-mediated selection, because pollinators can assess the variability of advertising and reward traits when they visit a sequence of flowers of the same plant. Hence pollinators can verify the reliability of plant signalling, and they can decide to leave or to continue foraging in a given plant based on this information [19]. By contrast, among-individual covariances are based on individual means, and thus they are statistical constructs that characterize populations, not individual phenotypes.

Disentangling within- versus among-individual sources of phenotypic variation requires the estimation of variance components, a task usually accomplished in ecological studies using mixed models [27]. We followed Dingemanse & Dochtermann [28] to disentangle the correlations between signals and reward from two hierarchical levels (i.e. within and among individuals). To this aim, we used Bayesian generalized linear mixed models (GLMM) with a bivariate response using the *MCMCglmm* package [29] of R software [30]. In these models, the reward (nectar sugar content) and signals (area of lower lip or area of corolla tube) were treated as a joint response, and individual identity was introduced as a random effect. The posterior distributions of two variance–covariance matrices were estimated from this model: (*a*) the variance–covariance matrix for the individual random effect, in which the diagonal corresponds to the variances of consistent individual effects in rewards and signals, and the off-diagonal value is the covariance between them; and (*b*) the residual variance–covariance matrix, which estimates the equivalent variances and covariances within individuals. Using the latter matrix, the within-individual correlation between signal and reward was estimated as the Pearson correlation coefficient.

Bayesian GLMMs were run for 130 000 iterations, with a burn-in of 30 000, a thinning interval of 100 and flat gamma-inverse priors [28]. For each correlation, we obtained the 95% highest posterior density (HPD) intervals. The posterior modes of the within-individual correlations were used in the comparative analyses. Two models were fitted per species: one where the joint response was the nectar sugar content and the area of the lower lip of corolla, and another in which the joint response was the nectar sugar content and the lateral area of corolla tube.

We assessed whether measurement error can affect estimates of correlation using a smaller set of 10 randomly chosen flowers per species. In these flowers, area measurements were taken three times as described above. For each species, we obtained three estimates of the correlation between nectar sugar content and frontal area of the lower lip, and three estimates of the correlation between nectar and tube area. We estimated, for each species and morphological measurement, the standard deviation of these three replicates, representing the variability owing to measurement error. In all cases, standard deviations of correlation coefficients were below 0.072. In addition, because the differences between the two estimated correlations (i.e. frontal signal–reward and lateral signal–reward) were important to test our evolutionary hypothesis, we estimated an ANOVA-based repeatability (R) statistic of this difference following Nakagawa & Schielzeth [27]. Repeatability was 99.2%, indicating that the proportion of the variance in correlation differences associated with measurement error ($1-R$) was very low.

(d) Phylogenetic analysis

The estimation of a phylogeny with branch lengths is required to test whether a character has evolved under drift or under selection with one or more adaptive optima. With this aim, we compiled from NCBI GenBank database cpDNA sequences (the intergenic spacer *psb*A-*trn*H and the fragment including the intron and second exon of *trn*L gene and the intergenic spacer *trn*L-*trn*F) from 70 taxa, involving 53 species from *Salvia* subgenus *Calosphace* and 17 outgroups. In addition, we generated the sequences for those species for which we measured the morphological and nectar traits, except for *S. longispicata*. PCRs were carried out using Qiagen multiplex PCR kits (Qiagen) with initial denaturation for 15 min at 94°C, followed by 35 cycles of 94°C for 30 s, 57°C for 1 min and 72°C for 1 min, and a final extension for 1 min. Sequences were aligned using MUSCLE [31], and verified by eye using SeaView [32]. Insertion/deletion mutations (indels) were coded manually following the simple method of Simmons & Ochoterena [33]. We estimated the phylogeny using a Bayesian approach as implemented in the program BEAST v. 1.7.5 [34], using a GTR + Γ + I substitution model and non-informative priors for all parameters. Three independent Monte Carlo Markov chains (MCMCs) were run for 50 million generations. Trees were saved every 1000 generations. Log files from each run were imported into Tracer v. 1.4.1, to examine effective sample sizes (ESS) and stationarity. This test suggested that the analysis had reached stationarity within 50 million generations and the ESS of all parameters exceeded 200. Tree files from individual runs were combined using LogCombiner v. 1.5.3 after removing 5000 trees from each sample. The maximum-clade-credibility tree topology was estimated from the posterior distribution of the trees (electronic supplementary material, figure S1). This ultrametric tree with branch lengths expressed in units of substitutions per site was pruned to include only the 18 taxa for which morphological and nectar data were collected. The corresponding internal branches were removed along with external branches during pruning.

(e) Comparative analyses

Before modelling the evolution of signal–reward correlations, we employ phylogenetic generalized least squares (PGLS) to characterize differences among pollination syndromes in the mean values of reward (nectar sugar content), signals (area of the corolla lower lip and area of corolla tube) and the predominance or not of frontal over lateral signals (i.e. area of the corolla lower lip minus area of corolla tube).

We modelled the evolution of within-individual signal–reward correlations under a BM process (which describes the neutral evolution of a quantitative trait) and under different OU processes (which describe different adaptive hypotheses corresponding to different selective regimens [22]) using the *OUCH* package for R [23]. This method uses maximum likelihood to estimate the strength of random drift (σ, the only parameter in BM models), the strength of selection (α) and one or more values of

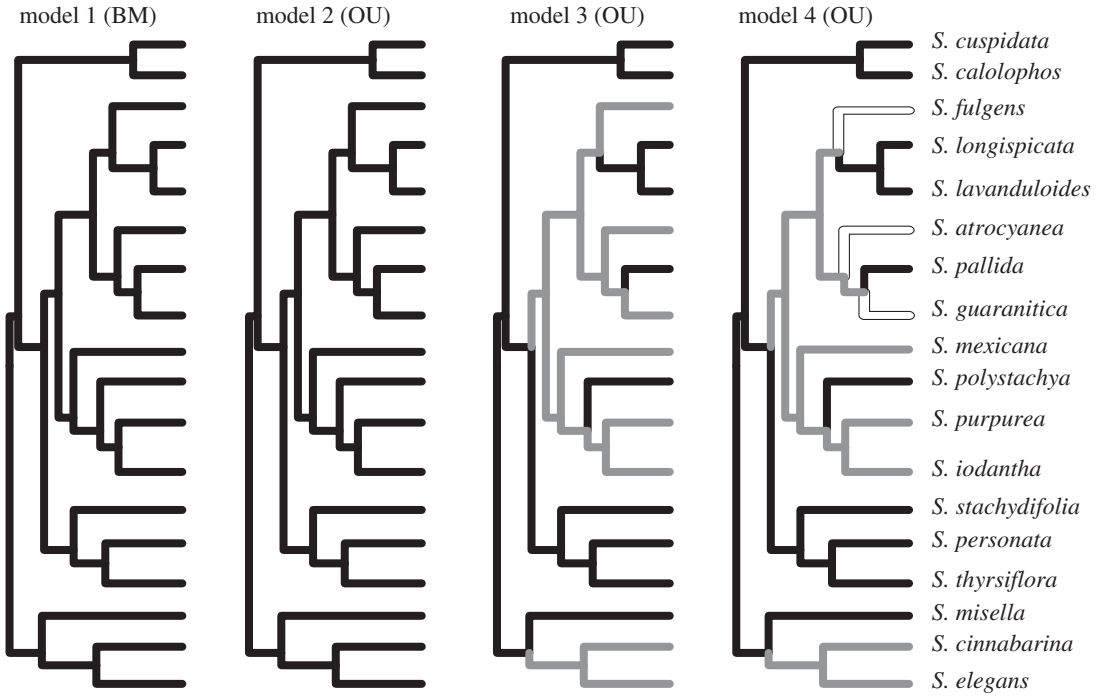

Figure 2. Evolutionary hypotheses about the evolution of within-individual signal–reward correlations. Model 1, BM model. Model 2, OU model with a single adaptive optimum. Model 3, OU model with two adaptive optima, corresponding with different pollination syndromes (grey, ornithophily; black, melitophily). Model 4, OU model with three adaptive optima (white, ornithophily and bilabiate flower architecture; grey, ornithophily and tubular or intermediate flower architecture; black, melitophily).

adaptive optima (θ). Bivariate models were used to allow for the correlated evolution of the two within-individual correlations under study. Four different models (figure 2) reflecting hypothetical selective regimens on within-individual signal–reward correlations were tested: in model 1, within-individual signal–reward correlations evolved following a pure drift (BM) process. In model 2, these correlations evolved under a single selective scenario following an OU process with global evolutionary optima (i.e. all species are attracted to a single adaptive peak in phenotypic space, but random drift simultaneously causes fluctuation around the optimum). In model 3, within-individual signal–reward correlations evolved under selection following an OU process towards two adaptive optima corresponding to different pollination syndromes, melitophily and ornithophily. Ancestral pollination syndromes were reconstructed by parsimony using the MPR function of the *ape* package in R [35], in order to estimate the selective regimes that historically operated on this group of plants. The 70 species of *Calosphace* included in the phylogeny as well as *S. californica* (as an outgroup) were used for reconstruction of ancestral states (electronic supplementary material, figure S2). Then, this tree with estimated ancestral states was pruned to include only the 18 taxa under study. Finally, model 4 hypothesized that signal–reward correlations evolved under selection towards three adaptive optima. Here, ornithophilous species were divided into two groups based on flower architecture following Wester & Claßen-Bockhoff [21]: 'bilabiate' and 'tubular or intermediate'. Ancestral regimen reconstruction was performed as described for model 3 (electronic supplementary material, figure S3). To compare the fit of the models, we used likelihood-ratio tests of each model against the BM model, the Akaike information criterion corrected for small sample size (AICc) and the Schwartz information criterion (SIC). We calculated 95% confidence intervals for the parameter estimates (σ, α and θ) of the best model using a parametric bootstrap with 10 000 simulations.

Phylogenetic uncertainty can affect our results, in particular because different topologies can result in different ancestral selective regime reconstructions. To explore the effect of this uncertainty, we repeated the entire process described above in 1000 trees sampled from the posterior distribution of phylogenetic trees [35] and compared the fit of the four evolutionary models. Thus, we obtained 1000 different log-likelihood, AICc and SIC values for each model, and used these values to examine the consistency of the best evolutionary model. Ancestral state reconstruction for some nodes can also give uncertain results, entailing a problem for the specification of OU models 3 and 4. Thus, we followed two alternative approaches to deal with this source of uncertainty in ancestral reconstruction: first, we arbitrarily assigned melitophily as the ancestral state of all the dubious nodes, because reversal from long to short flowers (i.e. from ornithophily to melitophily) is unlikely [36]; second, we randomly chose between ornithophily and melitophily, and assigned this arbitrary ancestral state to the uncertain node. Both procedures were applied to the comparison of evolutionary models in the 1000 trees sampled from the posterior distribution of phylogenetic trees. However, as they yielded almost identical results, we reported the results assuming that melitophily is the ancestral state in all dubious nodes.

3. Results

Hummingbird-pollinated species had, on average ($\pm$s.e.), 1.743 ± 0.372 µg more sugar in the nectar than bee-pollinated species ($t = 4.681$, $p = 0.0003$). Hummingbird-pollinated species had also larger corolla tubes than bee-pollinated species (mean difference $= 70.778 \pm 12.335$ mm^2, $t = 5.738$, $p < 0.0001$), but they did not differ significantly in the area of the corolla's lower lip (mean difference $= 25.618 \pm 13.590$ mm^2, $t = 1.885$, $p = 0.0777$). In all ornithophilous species except *S. atrocyanea*, the area of the corolla's lower lip was smaller than the area of the corolla tube, while the opposite pattern was evident in melitophilous species (mean difference in preponderance of frontal area $= 70.778 \pm 12.335$ mm^2, $t = 5.738$, $p < 0.0001$). Descriptive statistics of species means, and within-individual,

Table 1. Performance of BM model of evolution and OU models with one, two or three adaptive optima for within-individual correlations between signals and reward. Best model is in italics. ln, log likelihood; LRT, likelihood-ratio test for each model compared with the Brownian motion (BM) model; AICc, Akaike information criterion corrected for small sample size; SIC, Schwartz information criterion; d.f., model degrees of freedom.

	model 1 (BM)	model 2 (OU, global optima)	*model 3 (OU, two optima)*	model 4 (OU, three optima)
lnL	7.510	12.5903	*17.730*	17.984
LRT (*P*)	—	10.160 (0.017)	*20.493* (*0.001*)	20.948 (0.003)
AICc	−3.021	−3.847	*−6.660*	1.597
SIC	2.897	3.488	*0.375*	7.034
d.f.	5	8	*10*	12

Table 2. Parameters for the OU model with two regimes, corresponding to pollination syndromes: the strength of random drift (σ), the strength of selection (α) and the values of adaptive optima (θ). Ninety-five per cent CIs were obtained using parametric bootstrap. Parameters with a CI that does not include zero are in italics.

parameter	within-individual correlation between lower lip area and reward	within-individual correlation between corolla tube area and reward	correlated evolution
α	*9.572* (*2.259*; *297.612*)	*6.067* (*1.956*; *442.093*)	−3.198 (−215.923; 38.305)
σ	*0.686* (*0.018*; *10.251*)	*0.427* (*0.005*; *14.000*)	0.163 (−1.792; 4.112)
θ melitophily	*0.244* (*0.130*; *0.372*)	*0.154* (*0.039*; *0.271*)	—
θ ornithophily	0.148 (−0.001; 0.289)	*0.313* (*0.183*; *0.477*)	—

among-individual and total signal–reward correlations, can be found in the electronic supplementary material (table S3).

All selection models (OU) of the evolution of within-individual signal–reward correlations outperformed the drift model (BM; table 1; in all cases $p < 0.05$). The best model according to AICc and SIC scores was model 3, which explained the evolution of within-individual correlations among signals and reward using two adaptive optima corresponding to pollination syndromes. Under this model, the rates of adaptation (α) for the within-individual signal–reward correlations were greater than the corresponding drift parameters (σ) and significantly different from zero ($p < 0.05$) according to parametric bootstrap simulations. The α parameter for correlated evolution, however, was negative and non-significant, because its 95% CI included zero (table 2).

Posterior modes of species within-individual correlations clustered near the inferred adaptive optima (figure 3). The values of the adaptive optima (θ) were consistent with our hypothesis of within-individual signal–reward correlation evolution (table 2 and figure 3). The optima of hummingbird-pollinated species combine a low (and non-significantly different from zero; table 2) value for within-individual correlation between lower lip area and nectar, and a high value of within-individual correlation between corolla tube area and nectar, which indicates that the signalling function in ornithophilous species are accomplished by the corolla tube. Meanwhile, in bee-pollinated species both optima were significantly different from zero (table 2), but the optima for within-individual correlation between lower lip area and nectar was higher. Thus, in accordance with our expectations, signalling function in bee-pollinated species is likely to be accomplished mainly by the conspicuous lower lip of the corolla.

Different evolutionary scenarios yielded consistent results. Model 3 (OU with two adaptive optima corresponding to pollination syndromes) attained the lowest AICc score in 989 and the best SIC score in 933 out of 1000 phylogenies sampled from the posterior distribution. In addition, the mean AICc value from model 3 was significantly lower than the mean AICc value from the other models (in all cases Wilcoxon test $p < 0.0001$; electronic supplementary material, figure S4*a*), and similar results were obtained with SIC values (in all cases Wilcoxon test $p < 0.0001$; electronic supplementary material, figure 4*b*).

4. Discussion

It has been suggested that within-plant variation in floral trait expression could mask phenotypic differences among individuals, and hence could weaken selection pressures on floral evolution [1,3]. This expectation makes sense considering the evolution of average plant features: for instance, high within-plant variation in nectar could make it more difficult for a pollinator to differentiate among plants based on mean nectar production rates [1]. However, this rationale does not apply if the target of selection is within-individual variability in itself. This variation may influence plant fitness by altering the diversity and composition of pollinator assemblages, modifying overall attractiveness of plants to risk-averse pollinators or affecting the rates of geitonogamous pollination [6]. Thus, if within-individual variation and covariation among traits have a genetic basis, the adaptive evolution of these attributes could be expected. This work supports the hypothesis that within-plant signal–reward correlations have followed an adaptive evolutionary pathway, because it fulfils the prediction that hummingbirds and bees select on different associations between advertising and reward traits, in accordance with their differential use of lateral and frontal floral traits as signals [20].

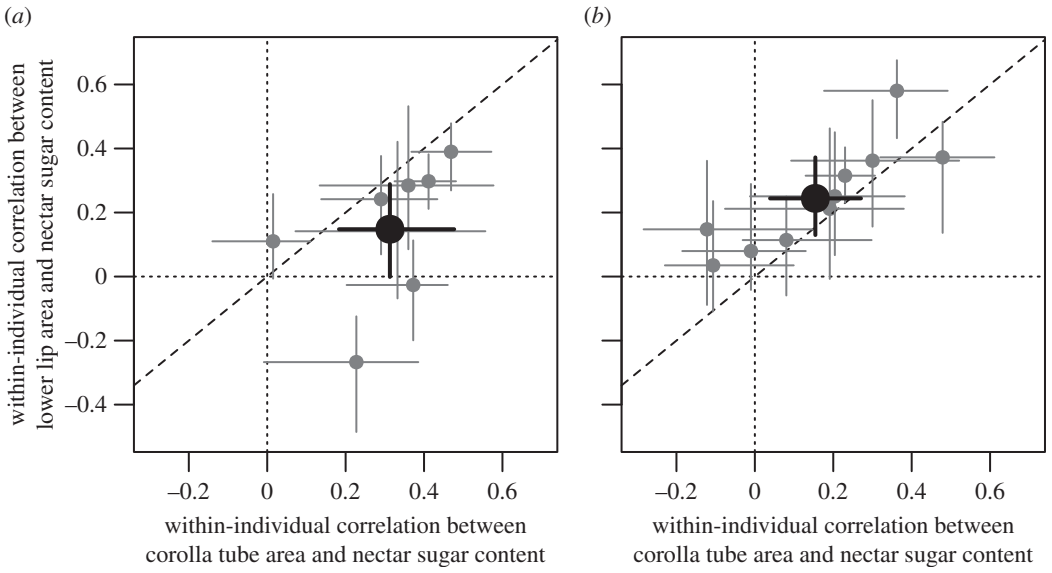

Figure 3. Morphospaces of within-individual correlations for (*a*) ornithophilous and (*b*) melitophilous *Salvia* species. Grey circles correspond to the posterior modes of within-individual correlations estimated by Bayesian GLMMs. Grey bars are 95% HPD intervals for each mode. Black circles represent adaptive optima estimated from an OU model of evolution with two regimens that correspond to pollination syndromes. Black bars are the 95% CI for these optima, estimated using 10 000 parametric bootstrap replicates. Dashed lines indicate identity. Dotted lines indicate zero correlations.

Alternatively, this evolutionary pattern could be a side effect of the modification of flower architecture during transitions from melitophily to ornithophily in Neotropical *Salvia* species. Because hovering pollinators like hummingbirds do not need a landing platform for foraging in flowers, evolutionary transitions to ornithophily often involve a reduction of the lower lip of the corolla [21,37,38]. This reduction may be achieved by decoupling the lower lip and other flower parts, and hence a relaxation in the correlation between the lower lip and nectar production rate. Our results, however, point to an inversion in the correlation patterns. Hummingbird-pollinated species not only have lower within-individual correlation between lower lip area and reward, but also show an increase in the correlation between corolla tube and nectar. Thus, our findings are consistent with the hypothesis of pollinator-mediated selection on within-individual correlations between different advertising traits and rewards. An open question is whether changes in pollination syndrome have driven further modifications in within-individual correlation patterns. In such cases, studies about phenotypic integration in modular organisms [12–14] should consider the within-individual source of variation in phenotypic traits.

Nectar production rate and flower length have undergone correlated evolution in hummingbird-pollinated species [39]. Although this correlation may be only a consequence of allometric relationships, this pattern is consistent with pollinator-mediated selection for larger flowers and high nectar production rate [39]. We emphasize the distinction between the correlated evolution of morphological and reward traits in flowers and the evolution of within-individual correlations. We did not test whether these traits experienced correlated evolution in *Salvia*. By contrast, we describe for the first time the macroevolution of within-individual correlations considered as complex phenotypes. Although there could be underlying causes such as developmental instability or reaction norms inside inflorescences [2,6], our results suggest that this complex phenotype could be a target of natural selection.

Our findings highlight the adaptive value of within-individual variation and covariation patterns, commonly disregarded as 'environmental noise'. We consider that within-individual association between signals and rewards constitute the basis of a subtle strategy that ranges from faithfully signalling plants to deceptive ones, and both extremes can be present within a single plant population [18]. Hence, these traits could be the source of adaptive evolution of different signalling strategies in plants. Evidence for different adaptive optima corresponding to different pollinator groups gives support for the adaptive role of these traits and opens new avenues for the study of the evolution of signal honesty in plants. This hypothesis should be investigated using, for example, manipulative experiments [11,40] on contemporary populations of *Salvia* species that are pollinated by both bees and hummingbirds. Future research should also investigate the genetic basis of within-individual associations between floral signals and rewards, and the behavioural responses of pollinators to changes in these variables.

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Data accessibility. DNA sequences: GenBank accession nos. KJ473958–KJ473991. Maximum-clade-credibility phylogenetic tree: TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2: S15364). Raw data: Dryad digital repository (doi:10.5061/dryad.841k6).

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References

1. Boose DL. 1997 Sources of variation in floral nectar production rate in *Epilobium canum* (Onagraceae): implications for natural selection. *Oecologia* **110**, 493–500. (doi:10.1007/s004420050185)
2. Diggle PK. 1997 Ontogenetic contingency and floral morphology: the effects of architecture and resource limitation. *Int. J. Plant Sci.* **158**, S99–S107. (doi:10.2307/2475170)
3. Williams JL, Conner JK. 2001 Sources of phenotypic variation in floral traits in wild radish, *Raphanus raphanistrum* (Brassicaceae). *Am. J. Bot.* **88**, 1577–1581. (doi:10.2307/3558401)
4. Thakar JD, Kunte K, Chauhan AK, Watve AV, Watve MG. 2003 Nectarless flowers: ecological correlates and evolutionary stability. *Oecologia* **136**, 565–570. (doi:10.1007/s00442-003-1304-6)
5. Ishii HS, Morinaga S-I. 2005 Intra- and inter-plant level correlations among floral traits in *Iris Gracilipes* (Iridaceae). *Evol. Ecol.* **19**, 435–448. (doi:10.1007/s10682-005-0896-1)
6. Herrera CM. 2009 *Multiplicity in unity: plant subindividual variation and interactions with animals*. Chicago, IL: University of Chicago Press.
7. Zhao Z-G, Du G-Z, Huang S-Q. 2010 The effect of flower position on variation and covariation in floral traits in a wild hermaphrodite plant. *BMC Plant Biol.* **10**, 91. (doi:10.1186/1471-2229-10-91)
8. Cresswell JE, Galen C. 1991 Frequency-dependent selection and adaptive surfaces for floral character combinations: the pollination of *Polemonium viscosum*. *Am. Nat.* **138**, 1342–1353. (doi:10.1086/285290)
9. Armbruster WS, Antonsen L, Pélabon C. 2005 Phenotypic selection on *Dalechampia* blossoms: honest signalling affects pollination success. *Ecology* **86**, 3323–3333. (doi:10.1890/04-1873)
10. Gómez JM, Bosch J, Perfectti F, Fernández JD, Abdelaziz M, Camacho JPM. 2008 Association between floral traits and rewards in *Erysimum mediohispanicum* (Brassicaceae). *Ann. Bot.* **101**, 1413–1420. (doi:10.1093/aob/mcn053)
11. Campbell DR. 2009 Using phenotypic manipulations to study multivariate selection of floral trait associations. *Ann. Bot.* **103**, 1557–1566. (doi:10.1093/aob/mcp032)
12. Armbruster WS, Di Stilio VS, Tuxill JD, Flores TC, Velazquez Runk JL. 1999 Covariance and decoupling of floral and vegetative traits in nine neotropical plants: a re-evaluation of Berg's correlation-pleiades concept. *Am. J. Bot.* **86**, 39–55. (doi:10.2307/2656953)
13. Ordano M, Fornoni J, Boege K, Dominguez CA. 2008 The adaptive value of phenotypic floral integration. *New Phytol.* **179**, 1183–1192. (doi:10.1111/j.1469-8137.2008.02523.x)
14. Boucher FC, Thuiller W, Arnoldi C, Albert CH, Lavergne S. 2013 Unravelling the architecture of functional variability in wild populations of *Polygonum viviparum* L. *Func. Ecol.* **27**, 382–391. (doi:10.1111/1365-2435.12034)
15. Blarer A, Keasar T, Shmida A. 2002 Possible mechanisms for the formation of flower size preferences by foraging bumblebees. *Ethology* **108**, 341–351. (doi:10.1046/j.1439-0310.2002.00778.x)
16. Healy SD, Hurly TA. 2001 Foraging and spatial learning in hummingbirds. In *Cognitive ecology of pollination: animal behavior and floral evolution* (eds L Chittka, JD Thomson), pp. 127–147. Cambridge, UK: Cambridge University Press.
17. Biernaskie JM, Walker SC, Gegear RJ. 2009 Bumblebees learn to forage like Bayesians. *Am. Nat.* **174**, 413–423. (doi:10.1086/603629)
18. Benitez-Vieyra S, Ordano M, Fornoni J, Boege K, Domínguez CA. 2010 Selection on signal–reward correlation: limits and opportunities to the evolution of deceit in *Turnera ulmifolia* L. *J. Evol. Biol.* **23**, 2760–2767. (doi:10.1111/j.1420-9101.2010.02132.x)
19. Broom M, Ruxton GD, Schaefer HM. 2013 Signal verification can promote reliable signalling. *Proc. R. Soc. B* **280**, 20131560. (doi:10.1098/rspb.2013.1560)
20. Dafni A. 1994 Note on side advertisement in flowers. *Func. Ecol.* **8**, 136–138.
21. Wester P, Claßen-Bockhoff R. 2011 Pollination syndromes of New World *Salvia* species with special reference to bird pollination. *Ann. Mo. Bot. Gard.* **98**, 101–155. (doi:10.3417/2007035)
22. Hansen TF. 1997 Stabilizing selection and the comparative analysis of adaptation. *Evolution* **51**, 1341–1351. (doi:10.2307/2411186)
23. Butler MA, King AA. 2004 Phylogenetic comparative analysis: a modelling approach for adaptive evolution. *Am. Nat.* **164**, 683–695. (doi:10.1086/426002)
24. Walker JB, Sytsma KJ, Treutlein J, Wink M. 2004 *Salvia* (Lamiaceae) is not monophyletic: implications for the systematics, radiation, and ecological specializations of *Salvia* and tribe Mentheae. *Am. J. Bot.* **91**, 1115–1125. (doi:10.3732/ajb.91.7.1115)
25. Dieringer E. 1991 Floral visitors and their behavior to sympatric *Salvia* species (Lamiaceae) in Mexico. *Act. Bot. Mex.* **13**, 75–83.
26. Lara C, Ornelas J. 2001 Preferential nectar robbing of flowers with long corollas: experimental studies of two hummingbird species visiting three plant species. *Oecologia* **128**, 263–273. (doi:10.1007/s004420100640)
27. Nakagawa S, Schielzeth H. 2010 Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biol. Rev.* **85**, 935–956. (doi:10.1111/j.1469-185X.2010.00141.x)
28. Dingemanse NJ, Dochtermann NA. 2013 Quantifying individual variation in behaviour: mixed-effect modelling approaches. *J. Anim. Ecol.* **82**, 39–54. (doi:10.1111/1365-2656.12013)
29. Hadfield JD. 2010 MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R Package. *J. Stat. Softw.* **22**, 1–22.
30. R Core Team. 2013 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
31. Edgar RC. 2004 MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **32**, 1792–1797. (doi:10.1093/nar/gkh340)
32. Gouy M, Guindon S, Gascuel O. 2009 SeaView ver. 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol. Biol. Evol.* **27**, 221–224. (doi:10.1093/molbev/msp259)
33. Simmons MP, Ochoterena H. 2000 Gaps as characters in sequence-based phylogenetic analyses. *Syst. Biol.* **49**, 369–381. (doi:10.1093/sysbio/49.2.369)
34. Drummond AJ, Rambaut A. 2007 BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* **7**, 214. (doi:10.1186/1471-2148-7-214)
35. Paradis E. 2012 *Analysis of phylogenetics and evolution with R*. New York, NY: Springer.
36. Whittall JB, Hodges SA. 2007 Pollinator shifts drive increasingly long nectar spurs in columbine flowers. *Nature* **447**, 706–710. (doi:10.1038/nature05857)
37. Cronk Q, Ojeda I. 2008 Bird-pollinated flowers in an evolutionary and molecular context. *J. Exp. Bot.* **59**, 715–727. (doi:10.1093/jxb/ern009)
38. Westerkamp C, Claßen-Bockhoff R. 2007 Bilabiate flowers: the ultimate response to bees? *Ann. Bot.* **100**, 361–374. (doi:10.1093/aob/mcm123)
39. Ornelas JF, Ordano M, De-Nova AJ, Quintero ME, Garland T. 2007 Phylogenetic analysis of interspecific variation in nectar of hummingbird-visited plants. *J. Evol. Biol.* **20**, 1904–1917. (doi:10.1111/j.1420-9101.2007.01374.x)
40. Weber MG, Agrawal AA. 2012 Phylogeny, ecology, and the coupling of comparative and experimental approaches. *Trends Ecol. Evol.* **27**, 394–403. (doi:10.1016/j.tree.2012.04.010)