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Summary

- Chemical communication is ubiquitous. The identification of conserved structural elements in visual and acoustic communication is well established, but comparable information on chemical communication displays (CCDs) is lacking.
- We assessed the phenotypic integration of CCDs in a meta-analysis to characterize patterns of covariation in CCDs and identified functional or biosynthetically constrained modules.
- Poorly integrated plant CCDs (i.e. low covariation between scent compounds) support the notion that plants often utilize one or few key compounds to repel antagonists or to attract pollinators and enemies of herbivores. Animal CCDs (mostly insect pheromones) were usually more integrated than those of plants (i.e. stronger covariation), suggesting that animals communicate *via* fixed proportions among compounds. Both plant and animal CCDs were composed of modules, which are groups of strongly covarying compounds. Biosynthetic similarity of compounds revealed biosynthetic constraints in the covariation patterns of plant CCDs.
- We provide a novel perspective on chemical communication and a basis for future investigations on structural properties of CCDs. This will facilitate identifying modules and

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biosynthetic constraints that may affect the outcome of selection and thus provide a predictive framework for evolutionary trajectories of CCDs in plants and animals.

Introduction

Chemical communication is ubiquitous and conveys information within and between cells and organisms. When compared with human language, an expression encoded in chemical information can be as simple as a 'STOP' traffic sign, in which a single word, or key molecule, contains all the information needed. Alternatively, chemical communication may require multiple molecules in the right proportions to transmit all of the necessary information, such as a sentence that reveals its meaning only through the syntax of multiple, well-selected words. The structure of and the functional components in complex communication systems, for example in human language or in bird songs associated with plumage coloration, have been identified by decomposing signals into individual components of either one or several sensory modalities (Sasahara *et al.*, 2012; Wilkins *et al.*, 2015; Levinson, 2016). Identifying universal characteristics of communication and complex displays that convey information facilitates the understanding of information production and perception, as well as of the development and evolution of communication systems (Schaefer & Ruxton, 2015; Levinson, 2016). Research on chemical communication has focused mostly on the mechanisms by which individual chemical compounds are produced (biosynthesis), perceived (chemoreception) or responded to (behaviorally) (Dudareva & Pichersky, 2006; Steiger *et al.*, 2011). By contrast, what we describe here as 'structural properties' of chemical signals – their chemical composition, patterns of covariation and effective proportions within complex chemical blends – are less frequently analyzed using comparative and meta-analytical approaches (Bruce *et al.*, 2005).

Chemical communication displays (CCDs), that is, blends of (volatile) compounds used to communicate with individuals of the same or other species (either as a cue or as signal), are usually complex compositions of few to well above a hundred compounds with various biosynthetic origins (Knudsen *et al.*, 2006; Wink, 2010; Wyatt, 2014). CCDs vary within a species both qualitatively and quantitatively (Kuppler *et al.*, 2016; Leonhardt *et al.*, 2016), and this variation may be perceived by the receiver as source of information and/or is a result of intraspecific genetic variation, or may represent plastic responses of the sender to the environment (Junker, 2016; Leonhardt *et al.*, 2016). However, variability in CCDs may be limited by biosynthetic constraints or by selective forces, by which the reliability of a signal would suffer from large variation. For example, variation in ratios of female sex pheromone blends has been shown to decrease their attractiveness to male moths, which suggests the action of strong selection on covariation patterns in some chemical communication displays (Löfstedt, 1990). Usually, the sender of the information produces a blend of organic compounds, whereas the receiver requires either a specific key compound (Sakurai *et al.*, 2004; Schäffler *et al.*, 2015) or a number of compounds present in specific proportions (Bruce *et al.*, 2005; Ozaki *et al.*, 2005; Bacquet *et al.*, 2015) in order to successfully perceive the information. Here, the concept of phenotypic integration is directly applicable. Specifically, for highly integrated CCDs, the proportional composition of compounds in bouquets would be conserved across individuals of the same species.

Phenotypic integration is a well-established tool in ecology and commonly is applied in morphological studies to infer functional adaptations and physiological constraints from patterns of covariation among traits in complex phenotypes (Pigliucci & Preston, 2004). Likewise, covariation and phenotypic integration in communication displays composed of several traits such as deer antlers, fruit color and morphology, and acoustic signals have been considered in order to evaluate, for example, their suitability to honestly signal physiological conditions or reward quality (Badyaev, 2004; Valido *et al.*, 2011; Blankers *et al.*, 2015). Correlations between quantitative traits (resulting in high phenotypic integration) may indicate functional modules which require a specific configuration to optimally perform or convey information, and thus are composed of traits that covary more strongly within than across modules (Wilkins *et al.*, 2015). Alternatively, correlations may result from pleiotropic, biosynthetic or developmental constraints with potentially no adaptive value (Berg, 1960; Pigliucci & Preston, 2004; Smith, 2016). Correlations between components of multimodal communication, in which visual, acoustic and/or chemical traits are jointly displayed, may indicate functional modules across modalities which are required for reliable and efficient communication and upon which selection can act (Hebets & Papaj, 2005; Smith, 2016). Blends of compounds such as plant scent bouquets, sex pheromones or hydrocarbons used as recognition cues in insects are similarly complex phenotypes (but unimodal, i.e. only olfactory cues are involved) and may also consist of modules (compounds that strongly covary in their amounts among individuals) revealing either functional or biosynthetic constraints.

In the present study, we aimed to identify patterns of covariation and biosynthetic constraints affecting variation and covariation within CCDs. Therefore, we assessed the phenotypic integration of CCDs (mainly plant scent bouquets and insect pheromones) in a comparative and meta-analytical approach. We tested for differences in the phenotypic integration of chemical communication between plants and animals (mostly insects), differences in plant vegetative and floral volatiles as well as the effect of herbivory on phenotypic integration of plant CCDs. Additionally, by applying approaches adapted from correlation network analysis, we defined modules of compounds with a pronounced covariation across samples. Finally, we tested the hypothesis that covariation patterns of compounds within CCDs (and thus the formation of modules and phenotypic integration) are a result of biosynthetic constraints by introducing a 'biosynthetic similarity index' for chemical compounds produced by plants. The 'biosynthetic similarity index' is based on the number of shared enzymes known to contribute to the biosynthesis of the compounds. In addition to these potential biosynthetic constraints on the covariation of compounds emitted by plants, we discuss potential

ecological and evolutionary causes of covariation of compounds by addressing the function and mode of action of CCDs and modules thereof. Our study provides a novel perspective on chemical communication and a predictive framework with which to identify ecologically and behaviorally relevant elements in CCDs, and thus a basis for future investigations of the phenotypic integration and modularity of CCDs. The framework and the methods proposed here to analyze CCDs may help in identifying modules and biosynthetic constraints that affect the outcome of phenotypic selection and may reveal different evolutionary trajectories and biosynthetic blueprints of chemical communication displays in plants and animals.

Materials and Methods

Chemical communication displays and phenotypic integration

We compiled datasets of organic compounds available in headspace or solvent extract samples of ≥ 8 individuals (in very few cases we used datasets with $5 \leq n < 8$ replicates) of a single species sampled within a study. A species list, references to the original studies and a brief summary of the sampling methods and the analyses of scent bouquets can be found in Supporting Information Notes S1. To calculate the phenotypic integration of chemical communication displays (CCDs), we followed a standard method commonly applied on morphological data that corrects for varying sample sizes (Wagner, 1984; Herrera et al., 2002; Perez-Barrales et al., 2007). For each species, we determined the Pearson's correlation coefficient r for all pairs of compounds (absolute amounts) produced by the species across the individuals and calculated eigenvalues of the resulting correlation matrix. The variance of the eigenvalues gives the integration index, a measure of the magnitude of phenotypic integration. To correct for varying sample sizes between species, the integration index (variance of the eigenvalues of the correlation matrix) was standardized by subtracting the expected value of integration under the assumption of random covariation (random covariation = (number of substances emitted by the species − 1)/number of samples; Wagner, 1984; Herrera et al., 2002) and then dividing by the potential maximum value of phenotypic integration in the given dataset, which is equal to the number of substances emitted by the species. The final result was multiplied by 100 to obtain the percentage of the maximum possible value, allowing comparison of the phenotypic integration values across species despite varying samples sizes and numbers of substances.

Our dataset included CCDs of plants and, within plants, the scent emissions of flowers, fruits, and leaves as well as of plant individuals that either experienced herbivory or served as a control with no herbivore contact. Additionally, animal CCDs (mostly insects) were included to characterize differences in plant and animal communication. To test for differences between phenotypic integration of CCDs of plants and animals, flowers and leaves, and control and herbivore-treated plants, we fitted linear mixed-effects models (LME4 package for R; Bates et al., 2015) with the phenotypic integration values as the response variable and the grouping variable as the explanatory variable (fixed factor). Because some species occurred two or more times in the dataset, we included species as a random factor. To account for different sample sizes of species we used the square root of the sample size as weight in the linear mixed-effects models, giving greater weight to phenotypic integration values that are based on larger sample sizes and thus are more reliable.

In order to test for phylogenetic signal in the phenotypic integration values of plant CCDs, we calculated Blomberg's K, which approaches zero with phylogenetic independence (Blomberg et al., 2003). The significance of K was tested using a randomization test implemented in the function PHYLOSIG in the R package phytools (permutation number = 999; Revell, 2012). We tested for phylogenetic signal in datasets based on the phenotypic integration values of flowers and leaves separately. The phylogeny for the included plant species was derived from an unparalleled time-scaled molecular phylogeny for 32 223 land plant species based on seven loci provided by Zanne et al. (2014) using the R package PEZ (Pearse et al., 2015).

Biosynthetic similarity of volatile organic compounds emitted by plants

In order to evaluate the biosynthetic basis of integrated CCDs, we selected a number of plant species with well-characterized scent bouquets and listed the enzymes involved in the biosynthesis of each compound. The following pathways were considered as entry points for the biosynthetic sequences leading to the different classes of compounds: 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway (monoterpenes, diterpene-derived compounds and tetraterpene-derived compounds; Eisenreich et al., 2001), mevalonate pathway (sesquiterpenes and sesquiterpene-derived compounds; Miziorko, 2011), shikimate pathway (aromatic compounds; Dudareva et al., 2013), lipoxygenase (LOX) pathway (fatty acid-derived compounds; Feussner & Wasternack, 2002; Matsui, 2006), leucine and isoleucine biosynthetic pathways (aliphatic compounds; Binder et al., 2007). Although most of the central enzymes in the different volatile organic compound (VOC) pathways are well described in the literature, a number of final enzymes modifying the core structures of the pathway products are unknown (in fact, 59 of 219 enzymes given in Notes S2 have been postulated). Such modifying reactions often comprise hydroxylation, the oxidation or methylation of hydroxyl groups, and the formation of esters. Because enzymes able to catalyze such reactions (e.g. monooxygenases, dioxygenases, O-methyltransferases, acyltransferases) are known from other biosynthetic pathways, we postulated them for the hypothetical reaction steps in VOC biosynthesis (Notes S3). For example, the last steps for the formation of lilac aldehyde are still unclear. This compound is presumably produced from the monoterpene linalool by at least one mono- or dioxygenase and an alcohol dehydrogenase and thus we added a hypothetical oxygenase and a hypothetical alcohol dehydrogenase to the end of the linalool pathway. Our biosynthetic analysis was focused on plant CCDs because insect CCDs are often composed of compounds that originate from a single or few precursors (i.e. from a

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single pathway). This limits the biosynthetic diversity of mixtures of compounds (Symonds & Elgar, 2008; Groot *et al.*, 2016) and thus also the variation needed for a statistical analysis. This approach resulted in a presence–absence matrix with individual compounds in rows and enzymes in columns. Based on this matrix, we calculated Sørensen distances between compounds. Small Sørensen distances indicate that a large proportion of shared enzymes are required in the biosynthesis of the compounds, whereas large Sørensen distances indicate few or no shared enzymes (compare with Barkman, 2001). We defined the mean Sørensen distances of the compounds present in a CCD displayed by one organ of one species as the biosynthetic diversity of the CCD, which is equivalent to indices expressing the functional diversity of communities (Gallien *et al.*, 2014; Junker *et al.*, 2015). The biosynthetic distance matrix for the compounds present in a CCD was compared with the correlation matrix of the same compounds (1 − Pearson's *r* of pairs of compounds, i.e. small values indicate high correlation of emission rates of substances across samples) using Mantel statistics based on Pearson's correlations. Correlations between the biosynthetic distance matrix and the correlation matrix (1 − *r*) indicate that biosynthesis likely controls covariation and thus phenotypically integrated CCDs.

Defining modules – covariation among compounds

Complex phenotypes are often composed of modules, which are composed of covarying traits (Wilkins *et al.*, 2015). To identify modules of compounds within CCDs, we adapted approaches from correlation network analysis (Langfelder & Horvath, 2008) using the correlation matrix (1 − *r*) for hierarchical cluster analysis (method UPGMA). Correlation networks were visualized using the software Cytoscape (Shannon *et al.*, 2003). We calculated the phenotypic integration and, if data were available (for plants), also the biosynthetic diversity within modules as described earlier.

Results

In total, we compiled $n = 3910$ chemical communication displays (CCDs, single samples) of $n = 37$ plant and $n = 19$ animal species (mostly insects) (Notes S1). On average, each species'/organ's CCD was characterized by $n = 30.1 \pm 6.8$ individual samples (mean ± SE, median = 10) and included $n = 37.8 \pm 2.3$ compounds (mean ± SE, median = 32.5). The mean phenotypic integration value *PI* across all sampled CCDs was $PI = 20.5 \pm 0.95$ (mean ± SE, Fig. 1). Plants usually had CCDs that were less
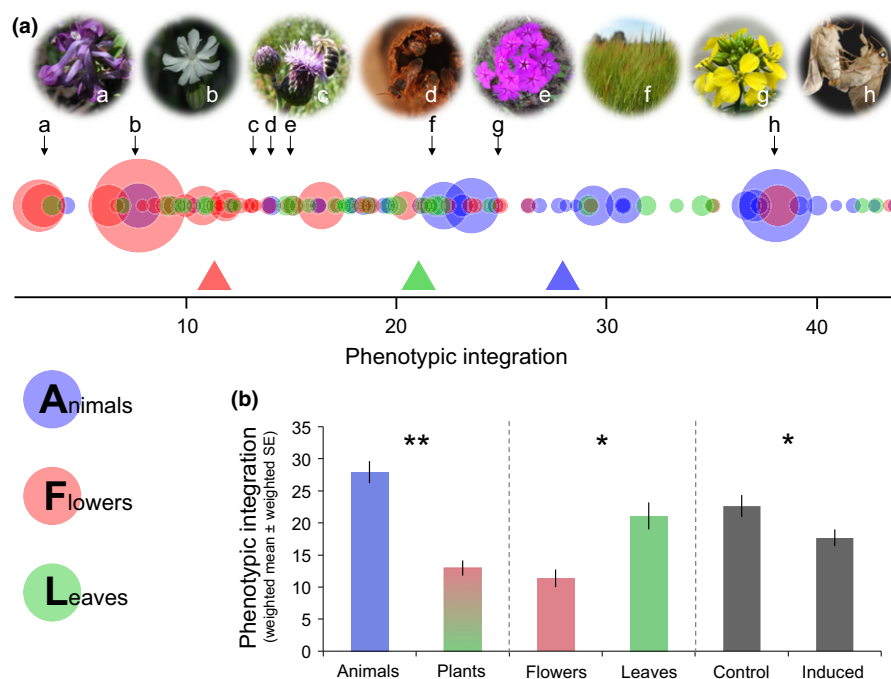

Fig. 1 Phenotypic integration of chemical communication displays (CCDs) of animals and plants. High integration values indicate a strong covariation of scent compounds within bouquets, that is, the relative ratio of emission rates of compounds is fixed across samples. (a) Phenotypic integration of scent bouquets emitted by animals, flowers and leaves. Circles are integration values of species; the size of the circles is proportional to the sample size (i.e. number of replicates) per species. Triangles are weighted means of phenotypic integration values with the square root of the sample size as weight. Pictures depict a selection of species included in the study (from left to right): (a) *Corydalis gotlandica* (photo credit: J. T. Knudsen), (b) *Silene latifolia* (S.Dötterl), (c) *Cirsium arvense* (R. R. Junker), (d) *Scaptotrigona pectoralis* (Dylan Burge), (e) *Phlox drummondii* (C. J. Majetic), (f) *Hordeum vulgare* (R. Glinwood), (g) *Sinapis arvensis* (R. Glinwood), (h) *Heliothis virescens* (A. T. Groot). The scale may serve as reference for future studies on the phenotypic integration of chemical communication displays. (b) Weighted mean ± weighted SE of phenotypic integration values with the square root of the sample size as weight of animals and plants, flowers and leaves, and of plants that emit herbivore-induced volatiles and those that did not experience herbivory before volatile sampling (control). Asterisks denote results of analysis of deviance based on linear mixed-effects models: **, $P < 0.01$; *, $P < 0.05$.

integrated than those of animals (analysis of deviance based on a linear mixed-effects model: $X^2_1 = 7.37$, $P < 0.01$; Fig. 1). Likewise, floral scents were less integrated than scent bouquets emitted by leaves ($X^2_1 = 4.01$, $P = 0.045$; Fig. 1) and plants that experienced herbivory emitted less integrated scent bouquets than plants that served as control with no herbivore contact ($X^2_1 = 4.04$, $P = 0.044$; Fig. 1). Scent bouquets emitted by fruits featured an intermediate integration value (PI $= 15.97 \pm 0.94$ mean $\pm$ SE; cf. Fig. 1), but the small sample size of fruit bouquets ($n = 13$ bouquets of $n = 3$ species) prevented further statistical analysis. Phenotypic integration values were independent of the number of samples per species (Pearson's product moment correlation: $t_{128} = -0.77$, $r^2 = 0.005$, $P = 0.44$) as well as of the number of compounds in the bouquet ($t_{128} = -0.37$, $r^2 = 0.001$, $P = 0.71$). We detected no phylogenetic signal in the phenotypic integration of CCDs, neither in the CCDs of flowers (Blomberg's $K = 0.04$, randomization test $P = 0.12$) nor in CCDs of leaves (Blomberg's $K = 0.39$, randomization test $P = 0.24$).

Biosynthesis of chemical communication displays and effects on phenotypic integration and modularity in plant chemical communication displays

Sørensen distances between compounds based on the number of shared enzymes required for the biosynthesis of the compounds presented a fair representation of the major pathways for volatile biosynthesis, namely the shikimate pathway, MEP pathway, mevalonate pathway, leucine and isoleucine biosynthesis, and lipoxygenase (LOX) pathway (Notes S2, S3). Plant CCDs were composed of volatiles derived from an average of 3.8 ± 0.31 major biosynthetic pathways involving 50.9 ± 4.2 enzymes (Table 1). The mean biosynthetic diversity (mean Sørensen distances between compounds based on shared enzymes) of plant CCDs was 0.65 ± 0.04 (mean $\pm$ SE, $n = 20$, Table 1). Biosynthetic diversity of compounds comprising a bouquet was independent of the number of compounds (Pearson's product moment correlation: $t_{23} = -0.37$, $r^2 = 0.01$, $P = 0.71$) but was positively correlated with the number of enzymes involved in the biosynthesis of the bouquet ($t_{23} = 2.3$, $r^2 = 0.19$, $P = 0.03$) and also with the number of major pathways involved in the synthesis of the compounds ($t_{23} = 4.55$, $r^2 = 0.47$, $P < 0.001$). The number of enzymes involved in the biosynthesis of the compounds comprising a CCD was strongly positively correlated with the number of compounds ($t_{23} = 6.1$, $r^2 = 0.62$, $P < 0.001$). The number of enzymes and the number of compounds was positively correlated with the number of major pathways involved in the synthesis of the compounds comprising a CCD (enzymes: $t_{23} = 7.27$, $r^2 = 0.70$, $P < 0.001$, volatiles: $t_{23} = 2.11$, $r^2 = 0.16$, $P = 0.046$). The phenotypic integration values were independent of the number of enzymes, the number of major pathways as well as the biosynthetic diversity ($t_{23} \leq 0.42$, $r^2 \leq 0.09$, $P \geq 0.68$).

Biosynthetic similarity of volatiles clearly correlated with covariation between pairs of volatiles in 17 of 20 scent bouquets for which information on both biosynthesis and covariation in emission rates of all (or most) volatiles was available (significant Mantel test comparing correlation matrix based on scent

emissions and matrix based on biosynthetic distances between volatiles; Table 1; Fig. 2). The nonsignificant correlations between the covariation matrix and the matrix based on biosynthetic distances (*Pinus sylvestris* and *Ipomoea purpurea*; Table 1) occurred in scent bouquets that were dominated by monoterpenes or sesquiterpenes, respectively. Monoterpenes and sesquiterpenes each share the same core pathway (the MEP and mevalonate pathways, respectively) and their biosynthetic routes often differ only in the final terpene synthase enzymes employed (Notes S2, S3). Therefore, the lack of a significant correlation may be a statistical artifact due to no or little variation in the biosynthetic distances between different monoterpenes and sesquiterpenes.

Modularity of chemical communication displays

On average, CCDs were composed of 5.28 ± 0.46 modules (mean $\pm$ SE, median = 3; Fig. 2) and 4.1 ± 0.29 (mean $\pm$ SE, median = 3) modules contained two or more compounds. On average, each module comprised 10.3 ± 0.78 compounds (mean $\pm$ SE). The number of modules was positively correlated with the number of compounds in a CCD (Pearson's product moment correlation: $t_{128} = 5.5$, $r^2 = 0.19$, $P < 0.001$) and negatively with the phenotypic integration value of the CCD ($t_{128} = -3.3$, $r^2 = 0.08$, $P < 0.01$). The phenotypic integration within modules was pronounced (43.6 ± 1.6 mean $\pm$ SE, median = 43.6; Fig. 2) and much higher than for the whole CCD (paired t-test: $t_{129} = -15.1$, $P < 0.001$). Accordingly, the mean biosynthetic diversity within modules was clearly lower (0.48 ± 0.23 mean $\pm$ SE, $n = 20$, Table 1) when compared with the biosynthetic diversity of whole plant CCDs (paired t-test: $t_{19} = -4.05$, $P < 0.001$). Neither the number of modules nor the mean phenotypic integration value within modules differed between plants and animals (analysis of deviance based on linear mixed-effects model: $X^2_1 \leq 0.81$, $P \geq 0.37$).

Discussion

Our analysis indicates that plants appear to utilize less integrated chemical communication displays (CCDs) than animals (mostly insects), but the CCDs of both plants and animals featured a broad range of phenotypic integration values. Thus, plants emitted CCDs with more variable proportions among the compounds of a bouquet than CCDs of animals that usually have more conserved ratios of compounds. Within plants, flowers emitted less integrated scent bouquets than leaves, and the integration of CCDs of both flowers and leaves decreased when the plants experienced herbivory. A unifying feature of all CCDs, however, was their modularity, meaning that CCDs were composed of modules of compound blends with stable ratios across samples of the same species. In the following, we suggest potential ecological, behavioral and biosynthetic explanations for these findings, and discuss implications for the evolution of CCDs. Although these different explanations are individually presented and are supported by different bodies of literature, they are not mutually exclusive and may

Table 1 Phenotypic integration and biosynthetic diversity, *bDiv*, of a selection of plant chemical communication displays (CCDs) included in the study

Family	Species	Organ	n	Bouquet					Modules			Mantel test	
				Compounds[2]	Enzymes	Classes	bDiv	Integration	Compounds[3]	bDiv	Integration	r	P
Amaranthaceae	*Beta vulgaris*	Leaves	19	13/11	55	4	0.80	34.57	5.50	0.68	46.98	0.61	**<0.001**
	Beta vulgaris	Leaves[1]	10	13/11	55	4	0.80	42.87	2.75	0.55	86.48	0.30	**0.045**
Asteraceae	*Achillea millefolium*	Flower	9	38/36	79	5	0.55	22.47	18.00	0.61	39.05	0.19	**0.047**
	Cirsium arvense	Flower	9	49/44	99	5	0.77	13.15	22.00	0.74	27.73	0.13	**0.003**
Brassicaceae	*Arabidopsis thaliana*	Flower	138	10/10	26	2	0.53	16.44	3.00	0.11	55.47	0.58	**0.017**
	Brassica nigra	Leaves	9	19/17	53	5	0.78	9.94	4.00	0.52	50.26	0.50	**<0.001**
	Sinapis arvensis	Flower	95	18/15	40	3	0.34	38.19	7.50	0.56	36.90	0.73	**<0.001**
Caryophyllaceae	*Silene latifolia*	Flower	98	28/27	52	4	0.68	6.32	2.88	0.30	46.16	0.34	**<0.001**
Convolvulaceae	*Ipomoea purpurea*	Flower	51	25/17	24	1	0.12	11.87	4.25	0.11	34.66	0.01	0.359
Fabaceae	*Vicia faba*	Flower	41	24/24	62	6	0.76	20.42	5.25	0.63	66.43	0.20	**0.011**
Papaveraceae	*Corydalis gotlandica*	Flower	109	28/26	65	5	0.74	3.20	2.56	0.52	56.26	0.15	**0.012**
Pinaceae	*Pinus sylvestris*	Leaves	16	25/25	41	2	0.41	20.06	8.33	0.24	44.95	−0.01	0.485
	Pinus sylvestris	Leaves[1]	17	25/25	41	2	0.49	15.12	2.71	0.16	64.74	0.08	0.227
Plantaginaceae	*Penstemon digitalis*	Flower	88	23/18	56	5	0.80	10.77	8.50	0.86	17.06	0.41	**<0.001**
Polemoniaceae	*Phlox bifida*	Flower	8	8/7	33	3	0.80	21.65	3.00	0.34	63.07	0.56	**0.017**
	Phlox carolina	Flower	8	26/25	67	5	0.82	18.54	5.00	0.63	61.94	0.27	**0.002**
	Phlox drummondii	Flower	31	21/17	55	5	0.72	11.95	2.33	0.25	67.94	0.27	**0.008**
	Phlox paniculata	Flower	9	22/19	52	4	0.76	18.48	4.75	0.72	42.52	0.41	**0.001**
	Phlox stolonifera	Flower	12	15/15	41	4	0.72	18.76	7.00	0.69	47.17	0.36	**0.005**
	Polemonium caeruleum	Flower	9	6/6	21	2	0.65	12.30	2.50	0.40	36.45	0.59	**0.003**

Given are: the number of samples *n* per species; the number of compounds; the number of enzymes and of major pathways (chemical classes) involved in the biosynthesis of the bouquets; the mean number of compounds per module; the biosynthetic diversity and the phenotypic integration value of the bouquets; and mean biosynthetic diversity and the phenotypic integration of the modules. Results of Mantel test based on Pearson's correlation testing for correlations between the covariation matrices based on scent emissions and distance matrices based on biosynthetic distances between volatiles are given. Significant correlations (bold *P*-values) indicate that the biosynthesis correlates with covariation patterns in emission rates of volatiles.
[1]Herbivore-induced volatiles.
[2]Number of compounds in bouquet/number of compounds with information on biochemical pathway.
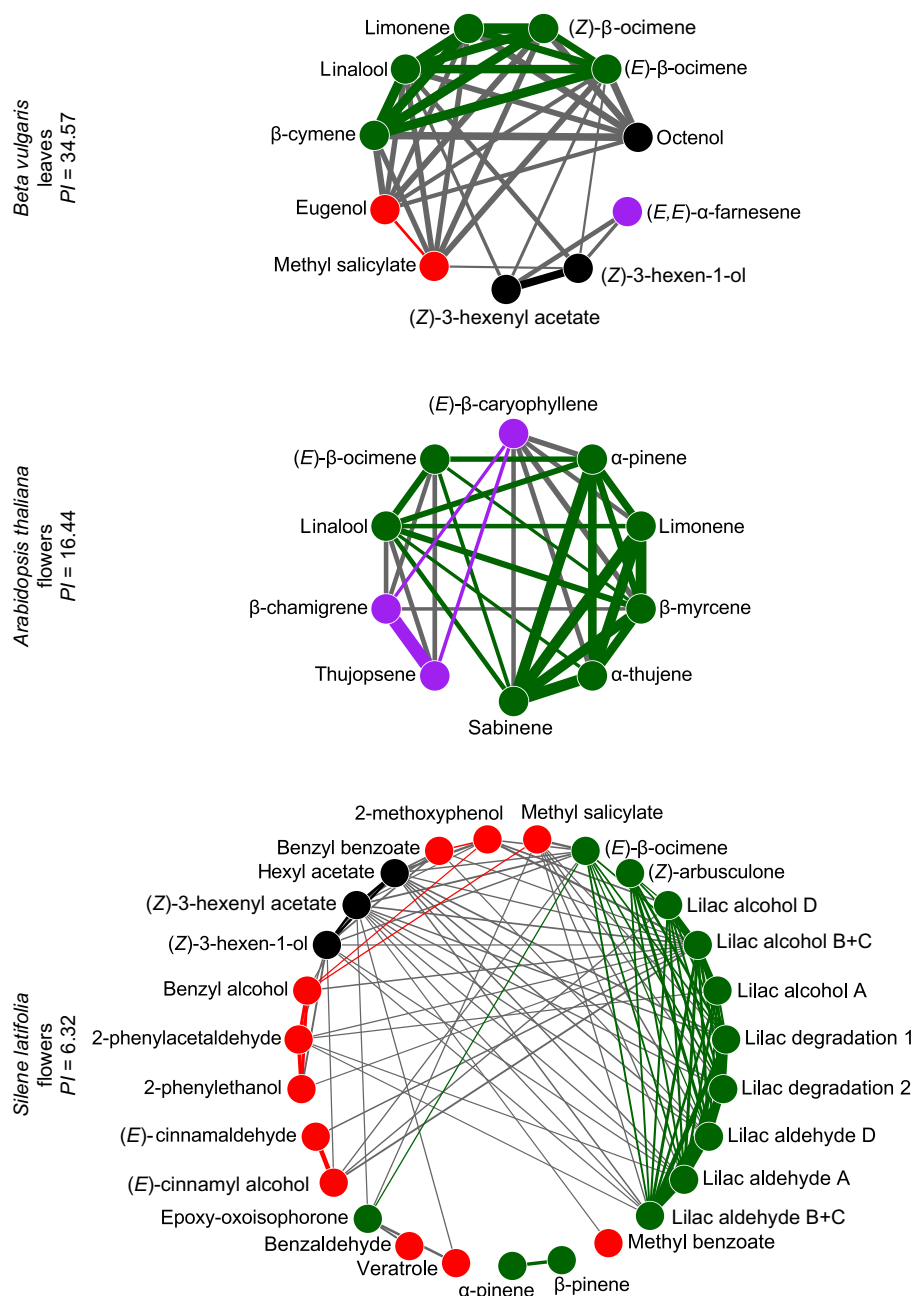[3]Mean number of compounds per module.

Fig. 2 Correlation networks of volatile organic compounds (VOCs) emitted by a selection of plant species included in the study. VOCs are shown as nodes. Width of edges is proportional to Pearson's correlation coefficient r, only significant correlations (Holm corrected) are shown as edges. Biosynthetic origin of VOCs and significant correlations between VOCs originating from the same pathway are color-coded with monoterpenoids in green, sesquiterpenoids in purple, aromatic compounds in red, and fatty acid-derived compounds in black. Significant correlations between VOCs originating from different pathways are depicted as gray edges. For each correlation network, the emitting species and organ as well as the phenotypic integration value of the whole bouquet are given. For additional information on the phenotypic integration of the modules as well as the biosynthetic diversity of the bouquets and the modules see Table 1.

equally and collectively contribute to covariation and integration of compounds within CCDs. However, the explanations from different lines of evidence clearly deserve experimental validation and our perspectives may stimulate future research testing these predictions.

Ecological and behavioral perspective on phenotypic integration in chemical communication displays

The mean differences in integration values between plants and animals may be explained by their functionality. For plants it often has been shown that one or a few key compounds within complex scent bouquets carry all the information required for a given interaction (Rasmann *et al.*, 2005; Riffell *et al.*, 2009; Bruce

& Pickett, 2011; Junker *et al.*, 2011; Schäffler *et al.*, 2015; Junker, 2016). Plant volatiles with different functions ranging from attraction of mutualists to the repellence of antagonists therefore constitute a multifunctional scent bouquet (Junker, 2016). Thus, in situations where a given function of a CCD is determined by one or few compounds, integration of the CCD can be low, meaning that the ratios of compounds can be variable without losing functionality. However, in cases where a number of compounds in fixed ratios is required for a given function, these compounds may be organized in modules with a tight covariation pattern whereas the other compounds of the bouquet may still vary independently from the compounds within the functional module, again resulting in a CCD with low integration values. The reduction in integration values in plants that

experienced herbivory and thus emit herbivore-induced volatiles may also be explained by the finding that a small number of volatiles within the blends have multiple functions, such as in the attraction of the herbivores' enemies or the repellence of the herbivores independent of the other compounds (Kessler & Baldwin, 2001; Gols *et al.*, 2011; Veyrat *et al.*, 2016).

In contrast to the importance of individual compounds in plant communication, information conveyance in animals (insects) often requires multiple compounds in specific ratios (Löfstedt, 1990; Symonds & Elgar, 2008; Bacquet *et al.*, 2015). For instance, recognition cues or sex pheromones in hymenopterans, where cuticular hydrocarbons have specific ratios, are used to discriminate between friends and foes (in nest mate recognition cues) or to identify mating partners (Kühbandner *et al.*, 2013; Leonhardt *et al.*, 2016). Another example is sex pheromones in moths: female *Spodoptera littoralis* moths emit a sex pheromone consisting of only two compounds (Z,E)-9,11-tetradecadienyl acetate and (Z,E)-9,12-tetradecadienyl acetate in a specific ratio of 99.5: 0.5 (Kehat & Dunkelblum, 1993; Hartlieb *et al.*, 1999). Although chemical communication in animals does not always require multiple components (e.g. Sakurai *et al.*, 2004), many species rely on fixed ratios of multiple compounds for intraspecific communication, which is reflected in the high integration values found in most animal CCDs. Note that pheromones also can exhibit considerable variation within and among populations (Groot *et al.*, 2014, 2016). Our data on animal CCDs mostly involve insect pheromones, which limits the potential for generalizations for the whole kingdom. Thus, future studies may provide a more comprehensive view on the structural properties of animal CCDs. In summary, differences in the integration of CCDs in plants and animals may be explained by different requirements on the composition of CCDs. Animals (insects) often communicate by means of fixed ratios of multiple compounds, whereas the scent bouquets of plants usually are composed of volatiles that each have distinct functions independent of the ratio to other compounds allowing for a multifunctional scent bouquet (Junker, 2016). Additionally, most animal CCDs we analyzed are signals for intraspecific communication, whereas plant CCDs represent mostly signals intended for members of other species, often more than one species. Thus, whether the receiver of a CCD is conspecific or not may have an effect on the phenotypic integration of CCDs, which, however, requires additional future investigations.

Biosynthetic perspective on phenotypic integration in chemical communication displays

Next to the ecological perspective as a potential explanatory approach for our results, biosynthesis may also, and simultaneously, underlie the phenotypic integration and modularity of CCDs. Our results show that biosynthetic similarities of compounds (defined by the proportion of shared enzymes in their biosynthesis) clearly correlate with their emission rates. In fact, compounds that share all or a large proportion of enzymes in their biosynthesis often covaried across samples of the same species. This suggests that the flux through a certain

pathway controls the total amount of pathway products to a large extent. In addition, the final reaction steps in the volatile-producing pathways are often catalyzed by enzymes, such as terpene synthases (Degenhardt *et al.*, 2009), that form a broad profile of products from a single substrate. Terpene synthases typically produce mixtures of compounds with fixed ratios and thus can also contribute to the covariation of production rates. For instance, β-chamigrene and thujopsene emitted by flowers of *Arabidopsis thaliana* are produced by the same terpene synthase, whereas (E)-β-caryophyllene is produced by a different terpene synthase (Tholl *et al.*, 2005), which is clearly reflected in the covariation pattern and the affiliation of the three sesquiterpenes to different modules within the scent bouquet of these flowers. Accordingly, the chemical diversity (i.e. the mean biosynthetic distance of the compounds) within modules was usually lower than in the whole CCD, suggesting that the formation of modules often results from biosynthetic constraints. Therefore, a biosynthetic explanation for the low integration in many plant bouquets may result from the high number of pathways involved in the biosynthesis of the CCD (Knudsen & Gershenzon, 2006; Wink, 2010), which results in highly integrated modules but poorly integrated CCDs. Insect pheromones or nest mate recognition cues, by contrast, often consist of compounds originating from a single or few pathways (Tillman *et al.*, 1999) resulting in integrated CCDs.

Phenotypic integration, modularity and the evolution of chemical communication displays

The modularity and integration found in CCDs have implications for the evolution of chemical communication. It has been discussed that the progress of ecological speciation, which often involves changes in CCDs in both plants and animals (Schiestl & Johnson, 2013; Bacquet *et al.*, 2015), is determined by the number of genetically (or in the context of this study biosynthetically) independent traits with no covariation (Nosil & Harmon, 2009). Modularity of CCDs reduces the number of independent traits and the number of modules may determine the effective number of selectable units. Studies on phenotypic selection of CCDs or other phenotypic traits often use multivariate models (e.g. multiple regression) to control for trait correlations (Lande & Arnold, 1983), and thus may identify a single trait (or chemical compound) under selection (Parachnowitsch *et al.*, 2012). Because such an identified compound can be part of a functional and/or biosynthetically constrained module, it will remain speculative whether the specific compound is the target of selection unless behavioral experiments (or other functional assays) support the finding. In research on multimodal communication it is recommended to test behavioral responses towards each component of a multimodal display as well as the response to the complete display in order to discriminate between redundant and nonredundant components or to detect additive or synergistic effects (Partan & Marler, 2005). In general, phenotypic integration and modularity may arise from functional, genetic (biosynthetic or regulatory), developmental or evolutionary causes, with multiple

interferences between these causes (Klingenberg, 2008). We have shown that biosynthesis can underlie covariation in CCDs and thus often define the modules indicating a pleiotropic regulation of the production of components within modules, which, however, may also facilitate the functionality of the CCDs (see earlier for the ecological/behavioral causes of integration). Thus, selection may sometimes act on modules, not on individual compounds, because the potential for independent variation of compounds within modules is limited. Although pleiotropy has been suggested to impede evolution, it may actually be adaptive if it allows for a coordinated evolution of compounds (organized in modules) (Smith, 2016).

Open questions and future directions

The present study provides insights into the structural properties of CCDs and discusses potential ecological, behavioral and biosynthetic causes for covariation in chemical compounds as well as phenotypic integration and modularity of complex compositions of these chemical compounds. Potential adaptive values as well as the biosynthetic constraints of (non-) integrated and modular CCDs remain speculative until appropriate experimental studies specifically address these issues. Therefore, our results and discussions may be a starting point and reference for future endeavors to analyze the structure of plant scent bouquets and animal pheromones and to explore the modular character of these CCDs. Further studies are needed to discriminate between genetic/biosynthetic constraints and a potential adaptive value of integrated CCDs and modules. Thus, in future studies correlation network analysis may help to understand variability in emission rates and to relate it to alternative ecological functions and biosynthetic constraints.

Another approach to test the adaptive significance of phenotypic integration in CCDs is to test for changes in covariation patterns in response to environmental conditions or biotic interactions. Our data show that, on average, scent bouquets of plants become less integrated after the plants experienced herbivory, which clearly indicates that biotic interactions interfere with covariation patterns. Herbivory often induces the emission of compounds that are specifically upregulated and are released to attract enemies of the herbivores or prevent future herbivore-attacks (Rasmann *et al.*, 2005; McCormick *et al.*, 2014; Veyrat *et al.*, 2016). Changes in the covariation pattern may destroy the search image of herbivores that locate their hosts by scent bouquets characterized by fixed ratios of compounds (Bruce & Pickett, 2011), which may suggest that changes in phenotypic integration are adaptive.

In order to discriminate between functional, genetic (biosynthetic), developmental or evolutionary causes of phenotypic integration and modularity (Klingenberg, 2008) in CCDs, we propose an approach suggested for morphological studies (Armbruster *et al.*, 2004). Patterns of variation and covariation of CCDs may be analyzed at the individual level (within genets, e.g. several flowers or leaves of the same plant individual), within and among population of species and finally among species (the latter may be hard to realize due to the often large qualitative variation of CCDs even between closely related species). Covariance of compounds in CCDs within genets results from ontogenetic effects, from environmental factors, or reflects developmental instability. The covariation within and among populations may largely result from pleiotropic effects or biosynthetic constraints, environmental factors or, in the case of among-population variation, also from selection or gene flow. Finally, covariation among species may result from pleiotropic effects or biosynthetic constraints, environmental factors, adaptive or stochastic speciation or natural selection and drift (Armbruster *et al.*, 2004). It is important to note that the pattern of variation and covariation at each higher level of the genetic hierarchy described here may be (statistically) influenced by covariation in lower genetic levels (Armbruster *et al.*, 2004).

The conceptual framework of phenotypic integration has so far mostly been applied to morphological traits (Pigliucci & Preston, 2004) and correlation network analysis has often been used in gene coexpression analysis (Langfelder & Horvath, 2008) or communication displays not involving chemical compounds (Wilkins *et al.*, 2015), but see Borges *et al.* (2013). Our analysis and results demonstrate that these concepts and tools are also applicable to chemical communication displays and aid the characterization of structural properties of these displays as well as the identification of potentially functional and/or constrained modules comprising strongly covarying compounds. Moreover, it illustrates major differences between the chemical communication systems of plants and animals (mostly insects) and we discuss potential ecological, behavioral and biosynthetic causes for these differences as well as for covariation patterns. Future studies will further contribute to a detailed understanding of the phenotypic integration in chemical communication displays and the implications for ecological and evolutionary processes. Thus, applying the concepts of phenotypic integration and modularity in chemical ecology provides novel perspectives and may facilitate a more comprehensive understanding of chemical communication.

Author contributions

R.R.J. conceived the research; R.R.J., T.G.K. and J.K. planned and designed the research; R.R.J and T.G.K. performed statistical analysis; R.R.J., T.G.K., J.K., L.A., J.D.B., R.M.B., N.M.vD., M.D., S.D., B.E., F.E., J.G., R. Glinwood, R. Gols, A.T.G., M.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Notes S1 Scent samples and methods to identify chemical communication displays.

Notes S2 Biosynthetic pathways of plant CCDs.

Notes S3 Volatile organic compounds and enzymes involved in their biosynthesis.

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