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Floral trait variation and integration as a function of sexual deception in *Gorteria diffusa*

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Phenotypic integration, the coordinated covariance of suites of morphological traits, is critical for proper functioning of organisms. Angiosperm flowers are complex structures comprising suites of traits that function together to achieve effective pollen transfer. Floral integration could reflect shared genetic and developmental control of these traits, or could arise through pollinator-imposed stabilizing correlational selection on traits. We sought to expose mechanisms underlying floral trait integration in the sexually deceptive daisy, *Gorteria diffusa*, by testing the hypothesis that stabilizing selection imposed by male pollinators on floral traits involved in mimicry has resulted in tighter integration. To do this, we quantified patterns of floral trait variance and covariance in morphologically divergent *G. diffusa* floral forms representing a continuum in the levels of sexual deception. We show that integration of traits functioning in visual attraction of male pollinators increases with pollinator deception, and is stronger than integration of non-mimicry trait modules. Consistent patterns of within-population trait variance and covariance across floral forms suggest that integration has not been built by stabilizing correlational selection on genetically independent traits. Instead pollinator specialization has selected for tightened integration within modules of linked traits. Despite potentially strong constraint on morphological evolution imposed by developmental genetic linkages between traits, we demonstrate substantial divergence in traits across *G. diffusa* floral forms and show that divergence has often occurred without altering within-population patterns of trait correlations.

1. Introduction

Phenotypic integration refers to the coordinated variation of morphological traits within functional modules, and is essential to the function of complex phenotypes [1,2]. Such integration of trait modules may result from shared genetic control in the form of pleiotropy, close genetic linkage and ontogenetic interaction [3,4]. Alternatively, trait covariation may reflect linkage disequilibrium built up by correlational selection on genetically independent traits [5–7]. While long-term selection for optimal functionality has likely promoted genetic and developmental integration [4], the extent to which these mechanisms constrain or promote further morphological evolution by natural selection is an important question in evolutionary biology [3,8].

Flowering plants comprise a developmental hierarchy of connected but discrete functional modules [9]. The reproductive structure of flowering plants, the flower, constitutes one such module composed of distinct units that function in the dispersal and receipt of pollen. In animal-pollinated plant species, strong pollinator-mediated selection pressure could favour the integration of floral traits to better fit the animal pollinator’s morphology and behaviour [6,10]. For example, the higher levels of covariation between corolla and filament length observed in *Raphanus raphanistrum* are likely due to selection imposed by pollinator behaviour [10]. The most common pollinators on this species
cannot enter the corolla tube, and so land on the petals and insert a proboscis. The position of the anthers relative to the corolla tube length must therefore be closely correlated: filaments too short and the anthers will not contact the pollinators, too long and pollen deposition might be inaccurate. However, in addition to this phenotypic covariation, flower modules are also subjected to genetic constraint, functional restraints and developmental linkage. Consequently, flowers, and flowering plants, have emerged as important systems in understanding the interplay of forces underlying phenotypic integration.

Berg [11], in her classic comparisons of trait correlations between plants with specialized and generalized pollination systems, laid the groundwork for exploration of patterns of floral trait integration in the context of plant pollination and breeding systems. Berg’s work suggested that the floral traits of plants with specific pollinators exhibit ‘correlation pleiades’ [11] or modules of integrated traits, whereas the floral traits of wind- or self-pollinated plants are less strongly correlated. Pollinator specificity was deemed to produce this pattern through floral adaptation for more efficient pollen transfer [11]. Further support for this idea has come from studies that found floral trait integration in pollinator-dependent outcrossing species relative to selfing species, which are less reliant on pollinators [12–14] and in pollinator-specialized versus generalized species [12,15,16].

However, several studies have also demonstrated equal or greater levels of floral integration in self-compatible versus self-incompatible species [12–14]. Pollinator-mediated selection may therefore weaken existing developmental genetic integration by relaxed selection or opposing selection on correlated traits [13,14]. Additional mechanisms must also maintain accurate pollen transfer within self-compatible species, contributing to floral integration independent of pollinator-mediated selection [12–14]. These mechanisms will include genetic linkage and pleiotropy, which can also constrain response to selection for trait covariance [17–19]. More random processes, such as genetic drift, may affect patterns of trait integration among populations [20], whereas environmental variation may alter patterns of covariance within vegetative versus reproductive units [21]. So patterns of floral trait integration are clearly complex [22] and influenced by diverse and contrasting processes, both extrinsic and intrinsic to the plant [23].

Although these influences have been examined in a broad range of species and experimental systems, few, if any, studies have specifically studied the role of pollinator-mediated selection on the integration of traits involved in pollinator attraction. Indeed, integration of traits involved in pollinator attraction has been suggested to be less likely [12], compared with the integration of floral traits involved in promoting accurate transfer of pollen to stigmas. In part, this is because attractive traits may not engender legitimate pollen transfer, and in part, because the same traits can also attract floral antagonists [24], leading to opposing selection for trait covariance. However, an exception to this idea might be species whose pollination is achieved through mimicry and sexual deception. Suites of mimicry traits are so specialized that male pollinators attempt to copulate with the female-mimicking floral structure within the vicinity of pollen bearing organs. Such precise mimetic structures may be less likely to indiscriminately attract floral antagonists, and more likely to be intimately associated with legitimate pollen transfer.

Stabilizing selection could therefore act on two aspects of these complex structures: (i) the mimetic structures that attract male insects and elicit the copulation response and (ii) the relative position of pollen-bearing structures for accurate pollen deposition on and pick-up from copulating males. Pollination by sexual deception is most prevalent in the Orchidaceae, and commonly in these sexually deceptive orchids, pollinator attraction has a significant olfactory component, including pheromone mimicry [25,26]. However, more recently a parallel system of sexual deception has emerged outside of the Orchidaceae, in Gorteria diffusa Thunb. (Asteraceae). Here, the primary component of attraction appears to be a mimetic suite of visual traits [27].

**Gorteria diffusa** is a self-incompatible annual from South Africa comprising at least 14 closely related, but morphologically discrete, allopatrial floral forms [28,29]. The system is characterized by variation in floral colour and the complexity and distribution of the black anthocyanin spots at the base of the ray florets (figure 1). These spots, which are complex phenotypic structures, play an important role in the attraction of the bee fly, *Megapalpus capensis* Wiedeman [30]. *M. capensis* is the main pollinator of all forms of *G. diffusa* [31]. Although both male and female flies visit and pollinate *G. diffusa*, males tend to prefer more complex spots [27] and often attempt to copulate with the spots of the most complex forms, resulting in increased pollen export [31]. The fly-mimicking spots typically contain specialized, three-dimensional papillate structures and UV reflective highlights that are important in deception, as they attract male, but not female flies [27]. Not all floral forms are engaged in sexual deception, however, and some forms interact with other aspects of *M. capensis* behaviour, such as sleeping and feeding activities (AG Ellis 2012, unpublished data). These features of the *G. diffusa* system result in a continuum of floral morphological variation set against a continuum in the extent of deceptive exploitation of pollinator mating behaviours. Using these juxtaposed gradients, we sought to evaluate floral integration and patterns of trait covariance in relation to sexual deception in *G. diffusa*.

We first explored the patterns of divergence of floral traits between *G. diffusa* floral forms. We were particularly interested in whether components of the insect mimicking ray floret spots exhibit most divergence between floral forms (in relation to other trait modules) as might be expected if selection has favoured increasing levels of deception, or alternatively, evolutionary transitions between non-deceptive and deceptive forms. We hypothesized that mating male flies impose stabilizing correlational selection on trait dimensions, which provide optimal mimicry. The floral mimicry of females would require the accurate relative positioning of multiple different spot traits to achieve a convincing pseudo female fly. We tested four predictions from this hypothesis: (i) that variance of the traits involved in insect mimicry should be lower than variance of non-mimicry traits in sexually deceptive floral forms; (ii) that traits associated with insect mimicry should be more strongly integrated than other traits; (iii) that increasing levels of deceit should be associated with tighter integration of fly-mimicking traits and (iv) that the most sexually deceptive forms should exhibit distinctive patterns of covariance between mimicry trait pairs in relation to non-deceptive forms. Finally, we explored possible genetic and developmental constraints on morphological divergence between floral forms, by comparing patterns of trait covariance across *G. diffusa* floral forms in relation to patterns within floral forms.
Figure 1. Ten floral forms within the G. diffusa species complex showing the full frontal image of the flower, and for each floral form, a close-up of the spotted floret. Floral forms with three-dimensional structures (a–g), floral forms without three-dimensional structures (h–j). Individual floral forms are as follows: (a) Buffels; (b) Spring; (c) Nieuw; (d) Koma; (e) Cal; (f) Okiep; (g) Garies; (h) Soeb; (i) Naries and (j) Oubees. Further descriptions of floral forms can be found in Ellis & Johnson [28].

2. Methods

(a) Taxon sampling
We sampled inflorescences from each of 10 floral forms of G. diffusa: Spring, Buffels, Nieuw, Koma, Okiep, Oubees, Soeb, Cal, Garies and Naries (figure 1). This selection spanned the range of sexual deception present in the G. diffusa system, from sexually deceptive forms eliciting strong copulation responses (e.g. Spring or Buffels, figure 1a,b), to floral forms eliciting high levels of mate-searching behaviours but no copulation (e.g. Cal, figure 1c), to forms that only induce feeding behaviours (e.g. Soeb, figure 1h). GPS coordinates for populations of each floral form were as follows: Buffels (S29.987, E17.659), Cal (S30.42, E17.936), Garies (S30.656, E18.054), Koma S29.915, E17.462), Naries (S29.701, E17.689), Nieuw (S31.372, E18.63), Okiep (S29.571, E17.868), Oubees (S29.917, E17.467), Soeb (S30.072, E17.587) and Spring (S29.694, E17.884).

(b) Measurement of floral traits
A single inflorescence was collected from each of forty individuals in each population. Each inflorescence was dissected to obtain: (i) a bird’s eye view of an entire spotted ray floret (figure 2(i)); (ii) a lateral image of the angle of spotted ray floret insertion (figure 2(ii)); (iii) a lateral view of a disc floret exhibiting the maximum visible pollen presenter exertion (figure 2(iii)) and (iv) an image exposing the length and width of the disc petal ends (figure 2(iv)). All inflorescence units were photographed at high resolution against a scale rule using a Nikon D90 SLR camera with an AF-S Nikkor 85 mm macro lens. Photos were analysed in ImageJ 1.46r (Rasband W. http://imagej.nih.gov/ij), and trait dimensions measured to the nearest 0.1 mm (or degree in the case of angles) by the same person. A total of 19 traits were measured for each inflorescence (see figure 2 for details). Two traits were angle measurements which were square-root-transformed prior to integration analyses. In addition, we measured head, thorax, abdomen and wing dimensions on 23 M. capensis individuals.

Spot traits (10 traits; e–n, figure 2(iii)(iv)), which are involved in visual mimicry of female flies [27], were treated as a separate trait module in analyses. Unlike the mimicry traits (spot traits) which are exclusively on ray florets, traits which are not directly involved in fly mimicry were measured across ray and disc florets. Because these divergent floret types likely experience differing genetic/developmental influences, non-mimicry traits were assigned to either a disc floret (five traits; a–d, figure 2(vi)) or a non-spot ray floret (four traits; a–d, figure 2(iii)) module. These three trait modules were treated separately in analyses. In some cases, traits could not be measured accurately from photos (e.g. owing to herbivore or dissection damage) in which case individuals were discarded from the dataset, resulting in sample sizes for trait modules ranging from 27 to 40 (mean = 37) individuals across floral forms. As far as possible, we sampled inflorescences at the same developmental stage (i.e. pollen presenters fully exerted in the outer whorl of disc florets) in order to reduce trait variance associated with ontogenetic variation. In both figures 1 and 2, the white circles are the UV highlights. The dark shaded areas in figure 2 are the areas of three-dimensional papillate structure, which are present in the darkly pigmented areas of some of the floral forms in figure 1.

(c) Quantifying the degree of sexual deception
While all G. diffusa floral forms are visited, and effectively pollinated, by the bombyliid fly M. capensis, they differ in the behavioural responses they elicit from the fly. All floral forms are visited by female and male flies feeding on nectar and pollen rewards, and differ primarily in the extent to which they elicit mating behaviours from male flies [31]. As we were primarily interested in the potential influence of sexual mimicry on floral trait integration, we quantified the degree of deception of floral forms as the proportion of visits by male flies involving mate-searching behaviour (copulation behaviour or inspection visits). Data were extracted from Ellis & Johnson [31], who presented arrays of G. diffusa inflorescences to male flies in cages and quantified their behavioural responses to different floral forms. Data were available for eight of the 10 floral forms used in this study and are presented in figure 3 (no data for the Oubees and Koma floral forms).

(d) Assessing trait divergence between floral forms
In order to determine whether traits differ between floral forms, we conducted a MANOVA with all traits as dependent variables followed by ANOVAs for each trait. In addition, discriminant function analysis (DFA) was used to determine how well the measured traits discriminate between floral forms and which
traits provide most discriminatory power. Two DFAs were conducted, one on a dataset including three-dimensional structure traits (a subset of floral forms) and the second on a dataset excluding all three-dimensional structure traits (all floral forms). Standardized factor coefficients were used to determine which traits contribute most to discrimination between floral forms on the first three axes recovered from the DFAs.

(e) Assessing trait variation and covariation within floral forms

To explore patterns of trait variation in the different floral forms, we firstly calculated coefficients of variation (CV) for all measured traits, which are comparable between traits with different ranges of values. We used a two-factor ANOVA to test for differences in trait variation among floral forms.
CVs across trait modules and between floral forms, treating measurements from individual traits as replicates. In addition, CVs of individual traits were compared using ANOVA, treating measurements from each floral form as independent replicates. Finally, correlations of CVs of all traits with degree of deception were assessed.

We calculated eigenvalues from principal component analyses of measured floral traits in each of the trait modules (spot traits, non-spot ray floret traits and disc floret traits) for all floral forms separately. We then calculated trait integration as the variance of the eigenvalues of the trait correlation matrices \[ \text{INT} = V (\lambda) \] [22] in each trait module for all floral forms. To control for differences in sample size between the floral forms investigated and between trait modules, we subtracted the expected eigenvalue variance under the hypothesis of random covariation of traits \[ \text{Exp(INT)} = \frac{\text{number traits} - 1}{N} \] from INT for each form to obtain the corrected INT values [32,33]. These were expressed as % of maximum INT. We used bootstrapping with 1000 repetitions to estimate the 95% confidence intervals (CIs) for corrected INT. Values were considered to represent significant integration if their 95% CIs did not include zero and significance of differences in integration between floral forms and trait compartments was assessed by examining overlap in CIs [20].

Patterns of covariation between traits were further explored by examining within-floral-form pairwise Pearson correlation coefficients and their significance. In addition, we examined pairwise trait correlations across floral forms using trait means for each floral form. These were compared with mean within-floral-form correlations in order to identify trait combinations where divergence has occurred counter to genetic/developmental constraints. To assess by examining overlap in CIs [20].

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(f) Assessing the influence of pollinator specialization and deception on trait integration

The influence of the degree of deception (proportion of mating/inspection behaviours elicited from Megapalpus males) on trait integration was investigated using ANCOVA with trait module as the categorical predictor and degree of deception as the continuous predictor. We were particularly interested in determining whether the influence of deception on trait integration varied between trait compartments (i.e. the interaction term in ANCOVAs). The influence of degree of deception on integration of traits in each of the three trait compartments was further explored using simple regression. All statistical analyses were conducted in \( R \) of \( \text{Stata} \).

3. Results

(a) Trait divergence between floral forms

Measured traits differed significantly between \( G. \, \text{diffusa} \) floral forms (MANOVA: all traits—\( F_{108,1211} = 76.7, p < 0.001 \); all traits excluding three-dimensional spot structures—\( F_{155,2564} = 51.79, p < 0.001 \)). DFA correctly classified 100% and 98.3% of individuals into floral forms based on the measured traits with and without three-dimensional structure traits, respectively. While univariate ANOVAs of all traits (electronic supplementary material, table S1) and MANOVAs of each trait compartment suggest that all measured traits differ significantly between at least some floral forms, standardized factor coefficients from the DFAs suggest that spot traits, and to a lesser extent non-spot ray traits, contribute most to the discrimination between floral forms. Distance to UV highlight, distance to three-dimensional structures and three-dimensional width (and to a lesser extent floret length and width and spot length and width) provide most discriminatory power on the first three axes of the DFAs of all traits including three-dimensional structures which account for 79.9% of between-group variance (figure 4a). Floret dimensions, spot dimensions, distance to UV highlight and ray floret presentation angle had highest coefficients on the first three discriminant functions (76.8% of between-group variance) from the dataset excluding three-dimensional structures (figure 4b).

(b) Variance of traits within floral forms

Contrary to our expectations, in a combined analysis across traits, trait CVs did not differ between floral forms, but differed significantly between trait compartments (trait compartment—\( F_{2,145} = 6.05, p < 0.01 \); floral form—\( F_{9,145} = 0.87, p = 0.56 \)). Furthermore, non-spot ray traits had significantly lower CVs on average than spot or disc traits. Moreover, analysis of CV differences between traits across floral forms (\( F_{18,158} = 27.4, p < 0.001 \)) showed that CVs of some visually attractive traits (UV length, UV width) were significantly higher than for other traits (table 1). Interestingly, while the position of the UV highlight on the spot has low CV, UV length and UV width (both position-independent measurements) have significantly higher variance. This indicates that the dimensions of this visually attractive trait are unlikely to be under strong selection in sexually deceptive floral forms. In fact, the CV values of all measured traits were not significantly correlated with degree of deception (all correlations not significant). Trait CVs of measured fly traits fell within the range of CVs of \( G. \, \text{diffusa} \) spot traits (fly traits—12.4–29.3%, mean = 19.3%; spot traits—8.7–60%, mean = 16.5%).

(c) Trait covariation within floral forms

Traits in all modules were significantly integrated with the exception of disc floret traits in the Soeb floral form (table 2). Apart from differences in disc floret integration between the Soeb and Oubees floral forms, integration of disc floret and non-spot ray traits did not differ significantly between floral forms. In contrast, integration of spot traits varied significantly across some floral forms (table 2). In all floral forms, mimetic spot traits were more tightly integrated than non-mimicry traits (disc floret and non-spot ray floret traits), but in the case of Soeb, Naries and Oubees, the trend towards tighter integration in spot traits was not significant. These patterns were supported by consistently high (0.61–0.93) and significant pairwise Pearson correlation coefficients between some spot traits (spot length, UV highlight position, three-dimensional structure position; table 3). In addition, these spot traits were strongly correlated with ray floret length, whereas other trait pairs exhibited weaker correlations.

(d) Trait covariation across floral forms

While the pairwise structure of trait covariation across floral forms largely matched mean within-floral-form correlations (table 3), there were important exceptions. The most notable mismatches in trait covariation patterns within and between floral forms involve correlations between the length of ray florets and the size of the spots and their component parts (table 3 and figure 5a). ANCOVA revealed that the slope of
the relationship between floret and spot length does vary across floral forms (interaction term: $F_{9,357} = 4.5, p < 0.001$), suggesting the potential for selection to have altered the patterns of covariation between spot and ray floret dimensions. In contrast, correlations between trait pairs determining the relative positioning of spot components (three-dimensional position, UV position) were consistent within and between floral forms. The obvious exception was spot width and three-dimensional structure width (figure 5b), which covaried significantly within floral forms, but not across floral forms. ANCOVA suggests that there is a consistent positive relationship between these traits across forms (figure 5b), which covaried significantly within floral forms, but not across floral forms. ANCOVA suggests that there is a consistent positive relationship between these traits (continuous predictor: $F_{1,243} = 98.5, p < 0.001$) and slopes of the relationship do not vary between floral forms (interaction term: $F_{6,243} = 0.87, p > 0.05$). Thus, divergence in the relative dimensions of these traits across forms has occurred without changes in the within-floral-forms relationship (i.e. the probable axis of genetic/developmental constraint). Closer examination of other trait pairs which exhibit clear divergence in mean values across floral forms, but have consistent correlation patterns within and between floral forms suggests that this pattern of divergence in relative trait dimensions across floral form, with no alteration of the within-floral-form trait covariation, is common. Examples shown in figure 5 include divergence between forms in the ratio of spot edge to middle spot length despite similar correlation of these traits within all floral forms (figure 5c; ANCOVA interaction term $F_{9,356} = 1.21, p > 0.05$), and divergence between the Spring and Okiep forms and the rest in relative positioning of UV and three-dimensional structures (both important for attraction of male flies) [27] on the spot despite similar within-floral-form correlation (figure 5d; ANCOVA interaction term $F_{6,243} = 0.72, p > 0.05$). In both these examples, major phenotypic shifts (UV highlights inside and outside of three-dimensional structures, curved versus flat spot edges) have occurred in opposition to the axis of least genetic/developmental constraint (i.e. the within-floral-form correlation), whereas additional divergence has occurred along the constraint axis.
Effects of sexual deception on trait integration

ANCOVA analysis showed that trait integration differs significantly between trait modules ($F_{2,18} = 10.27$, $p = 0.001$) and overall integration increases with degree of deception (proportion of mating/inspection behaviours elicited from *M. capensis* males; $F_{1,18} = 24.41$, $p < 0.001$). Importantly, the influence of deception on integration differs between trait modules (homogeneity of slopes tests interaction term—$F_{2,18} = 9.57$, $p = 0.001$). Simple regressions suggest that integration of spots and disc florets are significantly influenced by degree of deception, with spot integration being most strongly influenced (figure 6).

4. Discussion

Our data support the hypothesis that increasing exploitation of sexual deception for pollination increases integration of traits involved in insect mimicry in *G. diffusa*. However, the data suggest that integration does not result from the mechanism of stabilizing correlational selection acting on independent traits, because two predictions arising from this mechanism were not supported: (i) that the spot (mimicry) traits should have lower variance in sexually deceptive forms and (ii) that patterns of spot trait covariation, in particular the correlation slopes, should differ between floral forms under different selection regimes. Instead our data support the notion that selection for effective mimicry does not favour stable dimensions or absolute positions of individual spot components, but rather strengthens existing developmental integration between traits, achieving consistent relative positioning of the spot components involved in mimicry. In addition, our data suggest that divergence between the floral forms of *G. diffusa* has seldom involved uncoupling of the genetic or developmental links underlying trait covariance, but rather involves shifts in ratios between trait means across floral forms, i.e. we detect changes in the intercept, but not in the slopes, of the within-floral-form pairwise trait correlations. We develop these ideas in more detail below.

(a) Trait divergence between floral forms in *Gorteria diffusa*

Our analyses of trait divergence suggest that *G. diffusa* contains a number of discrete, easily diagnosed, floral forms, which differ in a range of floral traits (figure 4 and table 3). This confirms the findings of Ellis & Johnson [28], who described 14 floral forms in the complex. While these earlier analyses were based primarily on discrete characters, here we demonstrate that the same floral forms can be distinguished through detailed measurements of the position and dimension of floral traits across ray and disc floret trait modules. In addition, although we have not tested it here, Ellis & Johnson [28] showed that floral traits do not differ between greenhouse- and field-grown plants. We assume these findings extend to...
the traits measured here, suggesting that floral divergence between allopatric *G. diffusa* floral forms is unlikely to reflect environmentally induced plastic responses. Instead, variation in the degree to which forms exploit mating responses of their bee-fly pollinators (figure 3) is a likely driver of floral divergence in the complex [27,28,31,34] although alternative possibilities (e.g. genetic drift) cannot be completely excluded.

While we show that many aspects of floral and capitulum structure have diverged between *G. diffusa* floral forms, DFAs suggest that spot traits have diverged to a greater extent than other trait modules. This result is intuitive in that divergent selection imposed by mate-searching flies as opposed to feeding insects would act largely on spot traits as these are the important component of the mimicry signal. However, spot traits also differ across sexually deceptive forms suggesting that multiple trait combinations function effectively in fly mimicry. At this stage, it is unclear to what extent this pattern reflects polyphyletic convergent origins of deception, or whether it reflects subtle differences in selection pressures [35], or simply the random accumulation or sorting of mutations in different parts of the *G. diffusa* range.

(b) Trait variance and covariation in relation to sexual deception in *Gorteria diffusa*

In contrast to the clear divergence of trait means between floral forms, differences in trait variance and covariation are more subtle and complex, as is also evident in many other studies of floral integration [16]. Berg [11] and numerous other later studies [12,15,16] have analysed trait covariance within and among trait modules hypothesized to differ in selection imposed by pollinators. Using this same approach, we show that traits involved in bee-fly mimicry (the spot trait module) display higher levels of integration than other trait modules (table 2) and that integration of spot traits increases with the degree of sexual deception (figure 6).

Our analyses support the hypothesis that stabilizing selection imposed by mate-searching male flies on traits involved in accurate fly mimicry promotes spot trait integration. However, two lines of evidence suggest that stabilizing correlational selection on genetically independent traits, the classic mechanism suggested by Berg [11] and developed by others [10,36], is not the likely cause of increased trait integration in sexually deceptive forms of *G. diffusa*.

In the first line of evidence, we find no evidence for decreased variance of insect mimicry traits relative to other traits in strongly deceptive forms (table 1), nor decreased variance of spot traits in deceptive versus non-deceptive forms, which are clear expectations if stabilizing selection on individual traits is involved in generating covariance between them [37]. This finding indicates that the dimensions or absolute positions of individual spot traits are not under stronger stabilizing selection from mate-searching flies than other traits. However, alternative explanations for this result include the fact that we were unable to measure key traits such as the height and optical effects of papillae, which vary significantly across floral morphs (AG Ellis 2011, personal observation) and may be the dominant elicitors of the mating response, allowing more visual flexibility in other traits [28]. Or we are underestimating the degree of morphological specialization in spot traits required for pollination through feeding responses. However, we favour the first explanation, because integration resulting from correlational stabilizing selection is only likely when
Table 3. Phenotypic correlation coefficients (Pearson product-moment method) from pairwise comparisons of floral traits (i) within-floral forms (below the diagonal) and (ii) across floral forms (above the diagonal). Mean within-floral-form correlation coefficients are shown below the diagonal, and bold \* indicates trait pairs which were always significantly correlated, while bold represents those that were significantly correlated in ≥80% of floral forms. Correlation coefficients from pairwise comparisons of trait means across floral forms are indicated above the diagonal, and bold indicates significance at $p < 0.05$ without Bonferroni adjustment. Gray shading indicates the correlations between the traits within each of the three trait modules (left to right: non-spot ray traits, spot traits and disc floret traits). 3D, three-dimensional.
imposed by pollinators with consistent body sizes [11], for example, honeybees, where the brood cell determines size. The bee-fly *M. capensis*, which has an endoparasitic larval phase, exhibits substantial size variation (CVs for body dimensions are similar to those of spot traits) and thus mate-searching males are unlikely to select for specific female, and hence spot, dimensions.

In the second line of evidence, in all floral forms, strong spot integration results from covariation between the same suite of traits (table 3) and pairwise within-floral-form correlations exhibit remarkably consistent slopes (figure 5). This is the expectation if integration results from genetically or developmentally determined trait covariance. The alternative explanation, that correlational selection by pollinators is consistent across all floral forms, seems less plausible given the clear differences in pollinator behavioural interactions along the continuum from feeding to sexually deceptive forms.

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Further to previous studies that demonstrate an influence of pollinator specialization on integration of pollen transfer traits in animal-pollinated plants [12,15,16], our findings suggest selection for the tight integration of attraction traits in sexually deceptive flowers. Integration of attractive spot traits in *G. diffusa* appears to largely reflect genetic or developmental constraints, as has been suggested in several other systems [17–19]. But, in contrast to these studies, our data suggest that selection for attraction of mate-searching male flies in sexually deceptive forms has tightened existing genetic/developmental covariance of the key mimicry traits. In the absence of a deep understanding of the developmental genetic pathways underlying spot formation in *G. diffusa*, it is impossible to suggest precise mechanisms leading to tightened developmental genetic covariance. However, it is likely to include homogenization of cell response to intrinsic developmental signals operating across the floret or spot module, coupled with enhanced signalling and coordination between developmental pathways controlling individual spot traits [38].

Remarkably, disc floret traits, which are not involved in mimicry in *G. diffusa*, were also slightly more integrated in
Figure 6. The influence of degree of sexual deception (proportion of visits by *M. capensis* males involving mating or inspection responses as opposed to feeding responses) on trait integration in the three trait modules. R-squared values (*p < 0.05*) from simple regressions are shown. ANCOVA analysis indicates that degree of deception significantly influences integration when all trait compartments are considered, and that regression slopes differ significantly across trait compartments.

the most deceptive floral forms. This could simply be a consequence of selection for tighter developmental control in the spots if, for instance, some of the pathways of developmental regulation are linked across disc and ray floret modules. However, it might also be because successful exploitation of mating flies for pollination likely requires tighter integration of disc traits for pollen removal and deposition associated with more constrained pollinator behaviour. This second hypothesis is supported by the remarkable variation exhibited by the exertion of the pollen presenters. Although the higher variance of pollen presenter length may in part reflect ontogenetic variation between the individual inflorescences sampled, it is also notable that the two most sexually deceptive forms exhibit the highest mean length of pollen presenters (electronic supplementary material, table S1). Long pollen presenters could therefore be a component of sexual deception and qualitatively often appear to be positioned in close proximity to the ray petal spots (AG Ellis 2013, personal observation).

(c) Trait divergence, covariance and evolutionary allometry

Under the reasonable assumption that patterns of within-floral-form phenotypic trait covariance reflect the genetic variance covariance (G) matrix in *G. diffusa* floral forms, our data suggest that floral integration largely reflects covariance between key traits arising through shared genetic or developmental control. Contrary to the expectation that genetic/developmental integration should impose constraint on independent evolutionary divergence of the traits involved, we find in *G. diffusa* that the traits that co-vary most strongly are also those that are most strongly differentiated between *G. diffusa* floral forms (three-dimensional and UV position, spot and floret dimensions). Comparisons of within- versus among-floral-form trait covariation patterns (electronic supplementary material, table S1; table 3 and figure 5a) revealed three main patterns of trait divergence in relation to the axes of genetic/developmental constraint (i.e. correlation pattern within floral forms): (i) floral form trait means have diverged along the axis of constraint, (ii) while the correlation patterns remain unchanged, the ratio of trait means has changed (i.e. the intercepts, but not the slopes of correlations change across floral forms) and (iii) differences in the ratio of trait means arise in conjunction with changes in the slope of trait correlations.

The last pattern, involving changes in covariance patterns within floral forms, characterizes the most notable mismatches in trait covariation patterns within and between floral forms, namely correlations between the length of ray florets and the size of the spots and their phenotypic components (table 3 and figure 5a). It is evident that the mean trait ratios fall roughly into three allometric configurations: (i) small floret/large spot (sexually deceptive), (ii) large floret/small spot and (iii) large floret/large spot (feeding forms). Given the allopatic distribution of these floral forms, it is not possible to rule out that some of these slope differences are generated by different responses of similar genotypes to differing environmental conditions [39]. However, it is likely that most are allometric differences arising through genetic change for two reasons: (i) floral forms exhibiting convergent slope relationships occupy very different abiotic environments and maintain their phenotypes in common garden experiments [28] and (ii) different slope relationships seem to cluster between deceptive and non-deceptive floral forms. There is thus the potential for selection to have acted on spot length and floret length allometry.

However, this instance of altered spot-ray length allometry across the sexual deception continuum seems to be the exception. For all other spot trait pairs examined, we find divergence in trait means (and often their ratios) across floral forms despite unaltered allometric relationships between traits. This suggests that alteration of floral trait allometries is most often constrained by shared genetic/developmental control in *G. diffusa*, but that this has not necessarily constrained independent divergence of traits across floral forms. Under strong genetic/developmental constraint, selection for alteration of one trait should lead to correlated changes in linked traits thus restricting the regions of morphospace, which populations can occupy. Trait divergence between *G. diffusa* floral forms often follows this pattern (i.e. populations occupy different positions on a ridge in two-dimensional morphospace reflecting the axis of genetic/developmental constraint—figure 5b–d for examples). Perhaps more interesting is the fact that trait divergence patterns which generate the clearest morphological differences between floral forms (e.g. reversal of the relative position of three-dimensional and UV structures—figure 1b and f versus a,e,d,g; or flat versus curvaceous spot ends—figure 3) involve shifts in ratios of trait means away from the axis of genetic/developmental constraint in trait morphospace (e.g. figure 5c,d). Given the functional implications of these trait changes in the pollination context (e.g. exploitation of feeding versus mating behaviour for pollination), it seems likely that pollinator-mediated selection has driven independent divergence of traits despite strong genetic/developmental constraints and without altering these constraints.

5. Conclusion

Our study joins a body of literature that is consistent with Berg’s [11] hypothesis that pollinator-mediated selection
influences integration of suites of floral traits [12,15,16]. However, our findings contrast with previous studies in two main ways (i) we show that pollinator specialization through sexual deception influences integration of a suite of traits involved in pollinator attraction, whereas previous work has demonstrated this for traits involved in accurate transfer of pollen between anthers and stigmas but not attraction traits [12] and (ii) our data suggest that the influence of sexual deception on trait integration in *C. diffusa* does not often result from stabilizing correlational selection on genetically/developmentally independent traits, but rather reflects selection for tighter integration of existing genetically determined covariance. In addition, our data suggest that the remarkable diversification in floral form across *C. diffusa*, which likely arose through variable pollinator-mediated selection imposed along a gradient in levels of pollinator deception associated with exploitation of mate-searching male flies, has occurred despite potentially strong constraints imposed by genetic/developmental covariance between traits.

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