



REPLY

Species arguments: clarifying competing concepts of species delimitation in the pseudo-copulatory orchid genus *Ophrys*

RICHARD M. BATEMAN^{1,2*}, ELIZABETH BRADSHAW³, DION S. DEVEY¹,
BEVERLEY J. GLOVER⁴, SVANTE MALMGREN⁵, GÁBOR SRAMKÓ^{2,6},
M. MURPHY THOMAS⁴ and PAULA J. RUDALL¹

¹Jodrell Laboratory, Royal Botanic Gardens Kew, Richmond, Surrey, TW9 3AB, UK

²School of Plant Sciences, University of Reading, Whiteknights, Reading, RG6 6AS, UK

³John Innes Centre, Norwich Research Park, Colney Lane, Norwich, NR4 7RH, UK

⁴Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge, CB2 3EA, UK

⁵Radagatan 8, SE 531-52, Lidköping, Sweden

⁶Department of Botany, University of Debrecen, Egyetem tér 1, Debrecen, H-4010, Hungary

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We are surprised that our recent comparative study of the micromorphology of all species of the European orchid genus *Ophrys* L. (Bradshaw *et al.*, 2010) has attracted wide-ranging criticism (Vereecken *et al.*, 2011) from a well-established group of researchers who, for the sake of brevity, we will term ‘ethologists’ (ethology: ‘the science of character . . . ; the scientific study of the function and evolution of animal behaviour’). Our surprise stems from the decision of the authors to direct largely conceptual criticisms at a paper that is dominantly empirical, when we had in fact provided similar but more conceptually oriented targets that specifically addressed the systematics of *Ophrys* (e.g. Bateman, 2001; Devey *et al.*, 2008; Bateman *et al.*, 2003, 2010). Nonetheless, we welcome the opportunity to further explain the rationale underlying our views on adaptation, speciation and species delimitation in *Ophrys*.

The core argument from Vereecken *et al.* (2011) is that the perspective expressed in Bradshaw *et al.* (2010) and associated papers is ‘too simplistic and speculative and misrepresents the current state of understanding of *Ophrys* ecology and evolution’. We were able to distil from the text of Vereecken *et al.* (2011) eight specific criticisms. The first of these is not explicitly numbered here as it is merely contextual: they begin their critique by arguing that our paper overlooked important historical references regarding

issues such as the ecology and evolution of *Ophrys* and the colour vision of bees. Given that the paper of Bradshaw *et al.* (2010) spans 37 published pages and includes 110 references, it is not surprising that we were discouraged from adding further details or expanding our treatment of conceptual issues touched upon in the Introduction and Discussion of our paper. Selection of exemplar references was an inevitable constraint, although we were careful to cite reviews of the relevant historical literature (e.g. Kullenberg, 1961; Devillers & Devillers-Terschuren, 1994).

Of the remaining criticisms, enumerated below, the first is largely terminological, whereas the remaining six encompass genuine conceptual disagreements:

1. Our indefensibly broad usage of the term ‘co-evolution’ demonstrates that we have ‘misunderstood the widely accepted nature of the interactions between sexually deceptive orchids and their pollinators’, which in fact unilaterally favour the orchid.

We accept that we employed the term ‘co-evolution’ in an old-fashioned and undesirably lax fashion and that the more precise modern parlance advocated by Vereecken *et al.* (2011) is preferable; the term should indeed be restricted to rare situations where two interacting lineages exert selection pressure on each other. However, it should have been obvious to Vereecken *et al.* (2011) that we were fully aware of the underlying conceptual implications. All of our published papers acknowledge that the pseudo-copulatory

*Corresponding author. E-mail: r.bateman@kew.org

relationship between *Ophrys* spp. and their insect pollinators approximates behavioural parasitism of the insect by the orchid; for example, 'the insect gains little from this co-evolutionary [sic!] relationship, unless the naïve male's interaction with the flower is useful practice for the time when he finally meets a female of his own kind!' (Bateman *et al.*, 2010).

In fact, this suggestion of a positive impact on the fitness of the pollinator was not wholly frivolous, as is demonstrated by a contrasting example in which the orchid has a negative impact on its pollinator. Informed by his own studies of the Australian pseudo-copulatory orchid *Chiloglottis*, Schiestl (2005) noted that the orchid 'mimic' has a negative impact on the fitness of the insect 'model' if naïve males fail to learn to discriminate between the pheromones of the female insect and the pseudo-pheromones of the orchid, so that the wingless females consequently remain frustrated virgins. A less catastrophic reduction in fitness of male pollinators of *Ophrys* spp. could be incurred if dalliance with the flowers reduces their reproductive success (Vereecken, 2009; Vereecken & McNeil, 2010). In a discipline long plagued by 'Just So' stories, it is notoriously difficult to demonstrate conclusively that co-evolution is taking place in the present (e.g. Ridley, 1993) and arguably impossible to demonstrate that it has occurred in the past. Indeed, it is equally difficult to demonstrate that co-evolution has *not* occurred.

More generally, there exists a profound distinction between co-evolution and co-speciation – one that mirrors the distinction between microevolution and macroevolution. Both co-evolution and co-speciation are extremely challenging to demonstrate with confidence, but present evidence suggests that only rarely does the former lead to the latter. And it is the origin (i.e. speciation, macroevolution) and delimitation of species that are the primary subjects of our papers on *Ophrys*. Vereecken *et al.* (2011) are comfortable to extrapolate species boundaries from their ethological observations, whereas we view ethological data as providing, at best, a near-independent test of species boundaries that have already been drawn using less transient, and thus more reliable, intrinsic properties. This crucial distinction consistently informs our responses to the remaining criticisms of Vereecken *et al.* (2011).

2. Our ongoing uncertainty regarding the relative importance of olfactory, visual and tactile cues for pollinators of *Ophrys* is misplaced, as it has been demonstrated conclusively that the key to the attraction of male insects as pollinators is the floral scent.

We share the enthusiasm consistently shown by Vereecken *et al.* and many others for the admirably sophisticated pseudo-pheromonal systems exhibited by *Ophrys*, which have generated an extensive litera-

ture (e.g. Mant, Peakall & Schiestl, 2005; Ayasse, 2006; Schiestl & Cozzolino, 2008; Stöckl *et al.*, 2009; Vereecken *et al.*, 2011, and references therein) and have justly become a popular textbook case of pseudo-copulatory pollination and presumed floral adaptation (e.g. Raguso, 2001; Glover, 2007; Leins & Erbar, 2010). However, evolutionary biologists have long since learned the dangers of identifying a 'key' innovation in the absence of conclusive evidence. When a model evolutionary system has been explored in sufficient detail, it inevitably transpires that multiple innovations have accrued sequentially until reaching a critical mass of character-state acquisitions that permits an incontrovertible diversification event (e.g. Bateman *et al.*, 1998; Hunter, 1998; Donoghue, 2005), not least in the orchid family per se (Rudall & Bateman, 2002).

Many characters separate *Ophrys* from closely related genera (*Serapias* L., *Anacamptis* Rich. *s.l.*). Irrespective of whether phylogenies are constructed from morphological (Devillers & Devillers-Terschuren, 1994) or molecular (e.g. Bateman *et al.*, 2003; Devey *et al.*, 2008) characters, the genus *Ophrys* is subtended by a relatively long branch. In other words, much of the evolutionary history of the genus is unlikely to be represented among its extant species. Despite being comparatively simple, even the earliest-diverging extant species (most likely *O. insectifera*: Devey *et al.*, 2008) possesses the fundamental phenotypic innovations of the *Ophrys* flower. These include greatly reduced lateral petals, loss of labellar spur, acquisition of a highly heterogeneous labellar epidermis with papillate margins and a central speculum (Bradshaw *et al.*, 2010) and of course secretion of a cocktail of pseudo-pheromones (e.g. Schiestl & Cozzolino, 2008). Although the absence from the present-day flora of early-formed species of *Ophrys* prevents determination of the order in which these innovations were acquired, it seems likely that they emerged sequentially. And if, as we believe, Schiestl & Cozzolino (2008) were correct to suggest that the pseudo-pheromones were a pre-adaptive by-product of a routine biochemical pathway found in an ancestral 'ur-*Ophrys*', then it is likely that the pseudo-pheromones characterized the lineage from its inception. This means that pseudo-pheromones appeared too early in the evolutionary history of *Ophrys* to account for the present (in our view, modest) species diversity in the genus, which is more likely to reflect the more recent diversification in epidermal features thoroughly documented by Bradshaw *et al.* (2010).

In truth, given current evidence, the competing 'key' innovations identified by ourselves and by Vereecken *et al.* (2011) both fall within the purview of highly speculative 'Just So' stories. We do not share the confidence of Vereecken *et al.* that the many putative adaptations evident in the visual and tactile

features of the *Ophrys* labellum are unimportant relative to the pre-eminent pseudo-pheromones. The fact that a featureless stick bearing pseudo-pheromones is capable of attracting a potential pollinator is not sufficient to inform us whether pollination would be effected with a sustainable frequency of success in the absence of the visual and tactile cues. In short, there is more to the functionality of insect–pollinator interactions than merely capturing the attention of the insect. Moreover, classic adaptationist theory suggests that it is unlikely that the *Ophrys* flower would continue to invest heavily in its remarkable visual and tactile cues if they did not continue to contribute substantially toward improved pollination frequency, functioning as important elements of a suite of innovations that together provide a holistic pollination package.

3. Our speculation regarding the location and complexity of scent-emitting osmophores on the *Ophrys* labellum is unwarranted; rather, the alkanes that are a key element of the pseudo-pheromones of most *Ophrys* spp. ‘are not produced by osmophores, but by subcuticular cells in the epidermis of the plant tissues’, and thus that ‘the idea that osmophores may be a key for pollinator attraction in *Ophrys* reflects [our] lack of knowledge about the chemical nature of the behaviourally active ‘scent’ compounds’.

Given the extensive series of papers on the biochemistry of pseudo-pheromones published over the last half-century by Vereecken *et al.* (2011) and their predecessors, it is difficult to see how we could have remained ignorant of their empirical observations; we have long cited their admirable work. But neither are we ignorant of the nature and function of osmophores.

In her textbook of flowering plant anatomy, Rudall (2007) described osmophores as ‘modified floral structures that produce volatile secretions... typically possessing a relatively thick, domed or papillate epidermis with densely cytoplasmic contents’. Most secretory activity of all kinds (odours, nectar, stigmatic fluid) in angiosperms is the responsibility of the epidermal cells, even when they have become developmentally internalized into structures such as septal nectaries within a postgenitally fused gynoeceum (Rudall, 2002).

By definition, epidermal cells are subcuticular. Indeed, they are responsible for secreting the hydrophobic, lipid-rich cuticular layer that permeates the matrix of the thickened external cell walls and envelops the above-ground organs of all vascular land plants (e.g. Javelle *et al.*, 2011). We see no reason to exclude any relevant area of cuticle from being considered part of an osmophore, as it is laid down and repaired by the subjacent epidermal cells. Presumably, it is these epidermal cells that dictate any

alkanes emitted by the relevant areas of cuticle; if odour secretion was a more general property of the cuticle, potential pollinators of an *Ophrys* plant would be as readily attracted by its stem and leaves as by its flowers.

The scale attributed to an osmophore in different definitions ranges from a single cell to an entire organ, but it is most typically a cluster of phenotypically similar conical and often pigmented epidermal cells that release scent for pollinator attraction. We are currently comparing the properties of the conical–papillate cells that characterize the inner epidermis of the petals of *Ophrys* with those of many other angiosperms; such cells are specified by the MIXTA family of MYB-related transcription factors, which were first recognized in the model zygomorphic angiosperm *Antirrhinum majus* L. (Noda *et al.*, 1994).

Given the complexity of labellar micromorphology demonstrated in *Ophrys* by Bradshaw *et al.* (2010), it seems reasonable that we and others (e.g. Ascensão *et al.*, 2005) should express the desire for further research aimed at localizing specific biochemical functions, not least the emission of the pseudo-pheromones – a topic that has received surprisingly little attention thus far.

4. We presented only limited data on structural colour in *Ophrys* labella, overlooking the potential for high reflectivity of the speculum in the ultraviolet (UV) region and failing to demonstrate the presence of iridescence in the face of their own (unpublished) data.

Vereecken *et al.* (2011) are not the only authors in possession of relevant unpublished data. Bradshaw *et al.* (2010) was published as the first in series of papers comparing the labella of *Ophrys* spp. The primary aim of the paper was to use the now well-established molecular phylogenetic framework for *Ophrys* (Devey *et al.*, 2008, and references therein) as a basis for comparing the impressively complex labellum micromorphology of each of the ten identifiable lineages within *Ophrys*. In addition, the Discussion of Bradshaw *et al.* (2010) effectively constituted an outline of the collaborative Leverhulme-funded research programme that is currently underway in Kew and Cambridge. Far from considering only *Ophrys speculum* and *O. fusca* (Bradshaw *et al.*, 2010: fig. 18), as implied by Vereecken *et al.* (2011), we have produced transmission electron micrographs for each *Ophrys* species. The physical properties of the speculum are being explored in the Cavendish Laboratory, Cambridge, as an important element of a broader pan-angiosperm study of floral iridescence and reflectivity (properties that we clearly and carefully distinguish). Even brief scrutiny of our past publications would have reassured Vereecken *et al.* (2011) that we

already have a well-established track record in studying not only the physics of structural colour but also the optics and experimental behaviour of pollinating insects (e.g. Whitney *et al.*, 2009; Thomas *et al.*, 2010). In addition, we are actively researching the evolutionary-developmental genes that dictate the remarkable micromorphology of the *Ophrys* labellum, and are investigating (in collaboration with Queen Mary University of London) the possibility that the labellar epidermis constitutes a community of cells of contrasting ploidy levels.

It would have been neither desirable nor practicable to publish the results of all our present lines of *Ophrys*-related research within the already substantial and data-rich paper of Bradshaw *et al.* (2010). We hope that Vereecken *et al.* (2011) will eventually perceive our ongoing research programme as a source of data useful to their own, rather narrower, goals.

5. We have underestimated the degree of reproductive isolation that separates the microspecies of *Ophrys* and overestimated the frequency of hybridization between them.

We agree that a single pollination event 'has the potential to create several thousands of seeds'. *Ophrys* spp. generate larger numbers of seeds per capsule than most European orchids (cf. Arditti & Ghani, 2000: table 2), estimates ranging from 3000 in a plant of *O. apifera* Huds. to 60 000 in a plant of *O. sphegodes* Mill. (Molnár, 2011). However, one of us (SM) has examined and sown the contents of innumerable capsules of species, spanning the entire genus *Ophrys*, and concluded that individual capsules rarely contain more than 1000 genuinely fertile seeds. Moreover, this figure would, of course, be further reduced by any decrease in zygote fertility associated with hybridization. Scopece *et al.* (2007) found little decrease in postzygotic fertility for each of the several pairwise combinations of *Ophrys* macrospecies that they created. Admittedly, SM has detected greater variation in postzygotic fertility through a more extensive programme of crosses between microspecies. Nevertheless, he has succeeded through multi-generation crosses in combining the genomes of no less than seven macrospecies in a single reproductively competent lineage (five lineages are combined in Fig. 1A).

Vereecken *et al.* (2011) make an interesting point when they argue that 'any... transfer of pollen masses from one species of *Ophrys* to another has the potential to create several thousands of seeds, which in turn can result in hybrid individuals growing intermixed with their parents for several years.' The packaging of so many pollen grains in individual pollinia, and so many ovules in a single ovary, means that the phenotypic and genotypic composition of a small population can be largely dictated by a single random

pollination event that results in a single injection of vast numbers of hybrid seeds. This influx will easily override the averaging effects of small fitness advantages of individuals in large populations that are the cornerstone of microevolutionary theory.

Among European orchid genera, only *Serapias* shows as little average molecular divergence as the 'microspecies' of *Ophrys* (Bateman *et al.*, 2003). Vereecken *et al.* (2011) implicitly accept that some of the microspecies recognized by some traditional taxonomic authorities such as Delforge (2006) are of recent hybrid origin and hint that they might not choose to award to these entities the status of species. However, they also argue that much of the taxonomic diversity recognized by Delforge and others represents a large number of species that have a genuine right to recognition, despite the fact that they have not been isolated for a period sufficient to acquire fixed genetic markers. Moreover, this argument is applied by Vereecken *et al.* (2011) not only to data sets of popular neutral regions such as the internal transcribed spacer (ITS) but also to AFLPs, which in most bona fide species reveal substantial infraspecific variation in addition to interspecific variation. Primary hybrids are easily identified when their parents are not conspecific in our usage (i.e. have experienced a substantial period of reproductive isolation) because, by definition, their parents will possess contrasting molecular markers; the hybrid will inherit these markers from the seed parent and pollen parent but organelles will be inherited only from the seed parent. Even the products of recent introgression are generally readily identified through such analyses. However, if the parents have never acquired fixed molecular autapomorphies (i.e. by our definition they are conspecific, having experienced little if any reproductive isolation), there will be no genetic means of distinguishing a supposed hybrid from its supposedly non-conspecific parents. If this principle is rejected, the products of any sexual union are, by definition, hybrids, and the term loses any useful meaning.

Vereecken *et al.* (2011) attempt to rescue their tenuous position by concluding that 'although molecular markers may often show overlapping [sic] patterns between closely related species [sic], floral traits under selection, such as floral scent in *Ophrys*, may still be distinct (Mant *et al.*, 2005; Stökl *et al.*, 2009).' We assume that, by 'distinct', Vereecken *et al.* (2011) mean that the floral scent can operate as a fixed autapomorphy of a microspecies even if all other characters show evidence of ongoing or recent gene flow with other microspecies. We note that chromosomal inversions may significantly reduce the effects of introgression on some genic regions responsible for reproductive isolation in model animals (e.g. King,

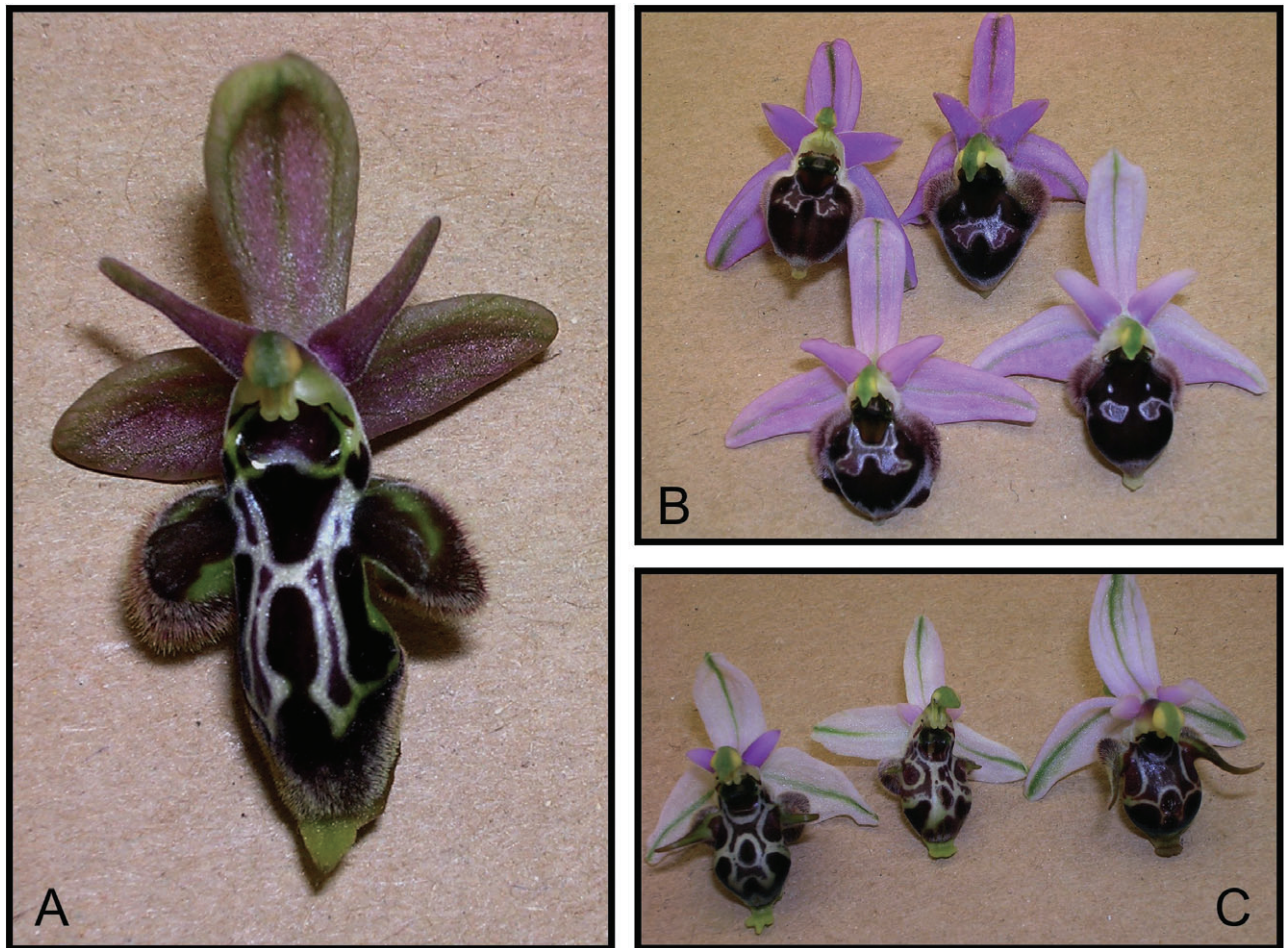


Figure 1. Systematic insights gained from captive breeding. A, fertile flower of an artificial hybrid that, through successive crosses, contains substantial genetic contributions from five of the ten macrospecies of *Ophrys*: *insectifera*, *tenthredinifera*, *speculum*, *fuciflora*, *cretica* (= *sphogodes* group). B, diverse phenotypes produced by self-pollination of a single flower of *Ophrys argolica* (*argolica* subgroup within *sphogodes* group). C, diverse phenotypes produced by self-pollination of a single flower of *O. cornuta* (*scolopax* group). Images: S. Malmgren.

1993; Noor, 2011). Nonetheless, we are not aware of any genetic mechanism that reliably and immediately preserves the ubiquity of a favourable allele in the face of substantial gene flow from individuals that lack that allele.

Indeed, we would extend this argument by asking whether the pseudo-pheromones are any less prone to infraspecific variation than any other feature of the *Ophrys* plant. In this context, much useful information can be obtained from experimental crossing experiments (Malmgren, 2008; Bateman *et al.*, 2010). In Figure 1B and C, we illustrate the flowers of groups of selected individuals, each group resulting from a single artificial self-pollination event, that nonetheless exhibit sufficient phenotypic variation to be assignable to multiple macrospecies. Although the individuals illustrated differ genetically only in any differences resulting from meiotic segregation and

crossing over, considerable phenotypic evidence can be discerned in characters such as speculum shape and epidermal micromorphology that we, and most other observers, regard as significant influences on pollinator behaviour. We suspect that these flowers would also have differed significantly in pseudo-pheromone content, especially as each *Ophrys* plant generates several pheromones (e.g. Mant *et al.*, 2005; Schiestl & Cozzolino, 2008; Stöckl *et al.*, 2009) and therefore presents several targets for genetic (or epigenetic) variation.

Potential explanations exist that suggest utility for such variation within populations. Given that at least two flowers must make physical contact with an insect before pollination is possible, there is a selective advantage to an orchid in delaying the point at which a naïve male learns to avoid a 'false female' flower (e.g. Schiestl, 2005; Ayasse, 2006; Paulus,

2006). Thus, within a population, rarer phenotypes on average induce a slower learning response than do common phenotypes and hence are selectively favoured, thereby increasing in relative numbers. Such negative frequency-dependent selection, an important component of Batesian mimicry, has previously been invoked in *Ophrys* (Schiestl, 2005; Vereecken & Schiestl, 2009). Although this selective mechanism encourages phenotypic diversity (Roy & Widmer, 1999), it must also compromise the population-wide fixation of selectively advantageous, pollinator-influencing characters; instead, it is a recipe for continual phenotypic fluctuations that are striking in the short term, but typically directionless in the longer term – in other words, microevolutionary rather than macroevolutionary.

6. Our series of studies of *Ophrys* is invalidated by our assumption that molecular markers are of ecological or taxonomic relevance; in the absence of ‘specific pollinator or pollen flow experiments . . . it is not possible either to infer gene flow between species or to discuss reproductive isolation and the specific taxonomic status of certain taxa’.

When addressing the critical issue of how best to gather and utilize molecular data for systematic studies of *Ophrys*, we take a far less polarized view than do Vereecken *et al.* (2011). We see considerable value in pursuing both molecular phylogenetic and population genetic approaches, and have spent much time considering how best to dovetail these empirically overlapping but conceptually distinct disciplines. In this context, Bateman *et al.* (2010) argued that, despite the existence of up to 30 named species concepts, only three primary criteria exist for circumscribing species:

- (1) Similarity of appearance of individuals: traditionally rooted in herbarium-based taxonomy, but now also offering the potential for instant molecular identification through DNA ‘bar coding’.
- (2) Reproductive isolation: the basis of the (in)famous biological species concept, rooted in estimates of gene flow within and between (meta)populations, based either on direct observations of pollen and/or seed migration.
- (3) Monophyly: the statistical circumscription of sets of individuals that consist of all the descendants of a presumed ancestor, based on shared morphological and/or molecular character states possessed by the plants under scrutiny.

Molecular techniques can be applied within each of these three frameworks. In each study, the overall workload is determined by pragmatic decisions regarding the number of plants per population and the number of populations per putative species to

sample plus the number of putative species to compare, balanced against the nature and number of the genic regions per plant selected for analysis in the light of rapid technological advances (Bateman, 2001, 2009).

In their taxonomically broad surveys of *Ophrys*, Devey *et al.* (2008) and Sramkó *et al.* (unpubl. data) adopted a conservative approach to species delimitation, requiring species to fulfill all three criteria. The resulting ten species are readily identified using either morphology or DNA bar coding (irrespective of which genome is selected) and are resolved as monophyletic by both molecular and morphological data sets. This admittedly conservative approach means that the species can be recognized and utilized by any competent user; specialist knowledge or equipment are not required. Any attempt to further divide these macrospecies that is based on adequate geographical sampling loses the support of at least one, and arguably all three, of the above criteria. Diagnostic characters, whether genotypic or phenotypic, are absent because their fixation (i.e. their elevation to the status of autapomorphies) has not been achieved within the relevant lineage.

Far from being irrelevant to assessment of reproductive isolation, as claimed by Vereecken *et al.* (2011), such studies are the only available method of examining reproductive isolation across the lineage and through evolutionary time. Finding autapomorphic molecular markers – nuclear and plastid, expressed and neutral – from a survey of an adequate sample of populations demonstrates conclusively that the lineage has indeed achieved an adequate level of isolation (considered in basic population genetic theory, its component populations are not experiencing an average influx of genes from two or more ‘foreign’ individuals per generation).

Vereecken *et al.* (2011) argue that such a survey might overlook a particular molecular autapomorphy that underpins a functional phenotypic autapomorphy that in turn confers reproductive isolation – for example, a unique addition to the pseudo-pheromone cocktail emitted by a particular population. We agree. For example, Bateman *et al.* (2010) stated that ‘if reproductive isolation precedes substantial genetic divergence of species by many generations (a period termed the “genetic divergence lag” by Bateman, 2009), a larger number of reproductively isolated species is likely to exist at any one moment in time than can be detected by standard genetic analyses.’ However, we went on to argue that it was a remarkable coincidence that 241 of Delforge’s (2006) 251 species presently occupy this evolutionary phase, which should in theory be brief and contain only a minority of species in any particular clade at any particular moment in time. Thus, the statement by

Vereecken *et al.* (2011) that, within *Ophrys*, 'it is likely that not all the narrow endemic species have a long history' would be rewritten by us as 'it is likely that few if any of the narrow endemic species have a long history.' Admittedly, even these sceptical statements open the possibility of expanding species recognition in *Ophrys* beyond ten. But how, in practice, can one distinguish between one of the few molecularly incohesive 'prospecies' that has a longer-term future from the plethora of transient prospecies that will never acquire genuinely diagnostic characters?

The high probability of rapid 'extinction' or re-assimilation of the nascent lineage is the primary reason why we do not regard direct observations of gene flow alone as an adequate basis for species circumscription. When conducted thoroughly, such studies can be admirably detailed and accurate, sometimes offering genuine insights into microevolutionary mechanisms. But these are observations made at a single (or, at best, very few) geographical locations at a single moment in time. They cannot possibly capture more than a modest fragment of the range of ecological and ethological influences experienced by the lineage as a whole through evolutionary timescales. This statement is especially true of interactions with pollinators, which vary not only from place to place but also at several timescales: diurnally, seasonally, yearly and beyond. Also, in contrast with genomes, there is no innate conservation mechanism for plant–pollinator relationships; they can change radically and instantly in response to (typically constantly fluctuating) ecological and environmental drivers. Vereecken *et al.* (2011) rightly note that 'Ophrys–pollinator interactions are flexible in time and space', but that is precisely why they are unsuitable as taxonomic characters per se, let alone as the predominant taxonomic characters that they advocate. Although highly focused studies of gene flow and pollinator specificity have the capacity to show that a lineage has *not* achieved reproductive isolation, they are wholly incapable of demonstrating that this *has* been acquired. Consequently, any attempt to convert such observations into species circumscriptions will be obliged to resort to authoritarian speculation (Bateman, 2009).

We emphasize that we do not subscribe to one of several available schools of thought, which argue that taxonomy should be pursued independently of evolutionary relationships and/or evolutionary mechanisms. Indeed, one of us (Bateman, 2011) recently advanced the highly controversial argument that prescribed base levels of morphological, molecular and ecological data should be internationally agreed before any further vascular plant species can be formally named – a viewpoint unlikely to prove popular among traditional taxonomists. Also, we have brought

to bear on these challenges our detailed systematic appraisals of several other orchid genera, including *Dactylorhiza* Neck. ex Nevski (Pillon *et al.*, 2007; Hedrén, Nordström & Bateman, 2011), *Gymnadenia* R.Br. (Bateman, 2006; R. M. Bateman *et al.*, unpubl. data) and *Epipactis* Zinn (Bateman *et al.*, 2005; Hollingsworth *et al.*, 2006). Nonetheless, none of us would support the suggestion of Vereecken *et al.* (2011) that the practice of taxonomic description should be postponed until a rigorous set of gene-flow data has been gathered for each putative taxon. Firstly, plant classification would effectively cease, as such data are presently available for, at most, 1% of the species of flowering plants that have been named over the last three centuries. Secondly, both logic and biology dictate that some means of establishing a taxonomic hypothesis of species circumscription using intrinsic data is essential before the extrinsic properties, such as habitat preference, geographical distribution and pollinator 'preference', can legitimately be characterized.

7. We have exaggerated the views of most ethologists regarding pollinator specificity; they 'are not systematically [wedded to] the extreme case of one orchid [equating with] one pollinator.' Rather, they support the view that 'Ophrys flowers are pollinated by a narrow taxonomic range of pollinators, from a single species to up to five closely related species', a diversity of plant–insect interactions that is sufficient to ensure that 'Ophrys–pollinator interactions are flexible in time and space.'

We find significant the fact that, when selecting three examples of *Ophrys* spp. that they accept entertain multiple pollinator partners, Vereecken *et al.* (2011) chose three abundant species. Of these, *O. sphegodes* and *O. lutea* Cav. span most of the Mediterranean; the third, *O. 'sulcata'* Devillers-Tersch. & Devillers, extends from Iberia to the former Yugoslavia and contains a significant proportion of the phenotypic variation encompassed by the legitimate macrospecies *O. fusca* Link. Thus, species that are widespread and abundant are considered by Vereecken *et al.* (2011) to entertain multiple partners, whereas 'species' that are local endemics must perforce be satisfied with a single partner. This apparent positive correlation between the areal distribution of a plant species and the diversity of its recorded pollinators is the pattern that one would obtain if one took a single widespread species and randomly divided its distribution into portions of radically different sizes. We would argue that, within the ten readily recognizable macrospecies of *Ophrys*, this is exactly the result that has been achieved, albeit unintentionally, by the last few decades of ad hoc species description pursued in the absence of a rigorous conceptual framework.

Naturally, we applaud the decision of Vereecken *et al.* (2011; see also Vereecken, 2009) to gradually distance themselves from the ‘one *Ophrys* species equates with one pollinator species’ argument made in some earlier ethological papers, instead moving towards a subtly different position of one *Ophrys* species equates with ‘one to five closely related [pollinator] species.’ The main argument advanced by Bateman *et al.* (2010) and Bradshaw *et al.* (2010) was conceptual; specifically, that such precise relationships would virtually preclude further speciation within *Ophrys*. However, the precision of plant–pollinator relationships is also challenged by empirical data. For example, when cultivated in western England and southern Sweden (S. Malmgren, unpubl. data), at least some microspecies endemic to regions of the Mediterranean appear to have no difficulty finding insect pollinators. For example, a hybrid individual of *O. apifera* × *scolopax* placed in the open in Malmgren’s garden set good seed in six of its ten flowers – a better success rate than the average achieved by allogamous *Ophrys* species around the Mediterranean (cf. Neiland & Wilcock, 1998).

Nevertheless, returning to our original conceptual arguments, we are surprised that Vereecken *et al.* (2011) are so keen to renounce the hypothesis that many *Ophrys* microspecies entertain only one pollinator and hence are highly likely to constitute evolutionary dead ends. Consideration of speciation patterns and processes in other thoroughly researched genera of European orchids suggests that a scenario of a few widespread species producing a larger number of less widespread and more specialized species is the norm rather than the exception. Within *Dactylorhiza*, the same pair of diploid macrospecies have crossed repeatedly during the Quaternary to generate a series of allopolyploid species that, inevitably, are similar (but, crucially, are not the same) in morphological features, molecular characteristics and ecological preferences (e.g. Pillon *et al.*, 2007; Hedrén *et al.*, 2011). In contrast, there is little evidence that each allopolyploid lineage has undergone subsequent dichotomous speciation events in which a novel species ‘buds off’ from the ancestral lineage. And within *Epipactis*, the widespread allogamous macrospecies *E. helleborine* has given rise to several lineages, again only subtly distinct in both morphological and genetic properties, that are autogamous and so are unlikely to undergo further speciation events through lineage dichotomies (e.g. Bateman *et al.*, 2005; Hollingsworth *et al.*, 2006).

All that is required to maintain considerable species-level diversity is that a few successful, and hence geographically widespread, species are sufficiently long-lived to periodically bud off shorter-lived

species. Seventeen years ago, one of us (RB) published a theoretical diagram showing exactly such a pattern of speciation and suggested that it was broadly applicable to all groups of vascular land-plants (fig. 8 of Bateman & DiMichele, 1994). That figure is reproduced here as the framework for Figure 2, as we consider the overall pattern to be applicable to *Ophrys*, even though the numbers of incipient speciation and hybridization events depicted are far too small. We have superimposed on this hypothetical diagram examples of different kinds of supposed *Ophrys* spp. *Ophrys sphegodes* represents the long-lived, geographically widespread, multi-pollinator lineages that we recognize as incontrovertible species. *Ophrys ‘garganica’* O.Danesch & E.Danesch is included as an example of a rare taxon endemic to the island of Crete that is likely to reflect a relatively recent ‘budding’ event, while the similarly rare *O. ‘homeri’* M.Hirth & H.Spaeth, endemic to the Aegean island of Chios, is considered by us to represent a few populations reflecting recent hybridization between the *O. sphegodes* and *O. fuciflora* (F. W. Schmidt) Moench macrospecies.

We assume that the ethologists would readily accept the ten macrospecies of *Ophrys* recognized by us (cf. Devey *et al.*, 2008; Bateman *et al.*, 2010). We are unclear of their position regarding ‘species’ that are of recent hybrid origin and thus usually transient; our impression is that at least some ethologists would not regard such populations as bona fide species, but that they believe that such populations are much less frequent than we have suggested. If so, this places the main emphasis on how best to identify a transient hybrid population. We suspect the main disagreement between ourselves and the ethologists concerns the second category of putative species; those such as *O. ‘garganica’* that could be interpreted as recent attempts at divergence from their widespread ancestral lineage. If these have arisen through positive selection or drift, they are of necessity only subtly distinct from their ancestral lineage, both genotypically and phenotypically, whereas a saltational origin would allow a more radical phenotypic (but not genotypic) shift (e.g. Bateman & DiMichele, 1994). Irrespective of underlying evolutionary mechanism, in all cases, the novel lineage would initially have a local distribution and thus be comparable with many of the microspecies recognized within *Ophrys* by, for example, Delforge (2006).

However, extrapolating from both first principles and our own field experience, we would argue that almost all such ‘prospecies’ are extremely transient in the context of evolutionary timescales; most populations rapidly succumb, either becoming extinct or being reabsorbed into their long-lived parental lineage through ‘hybridization’. To this extent,

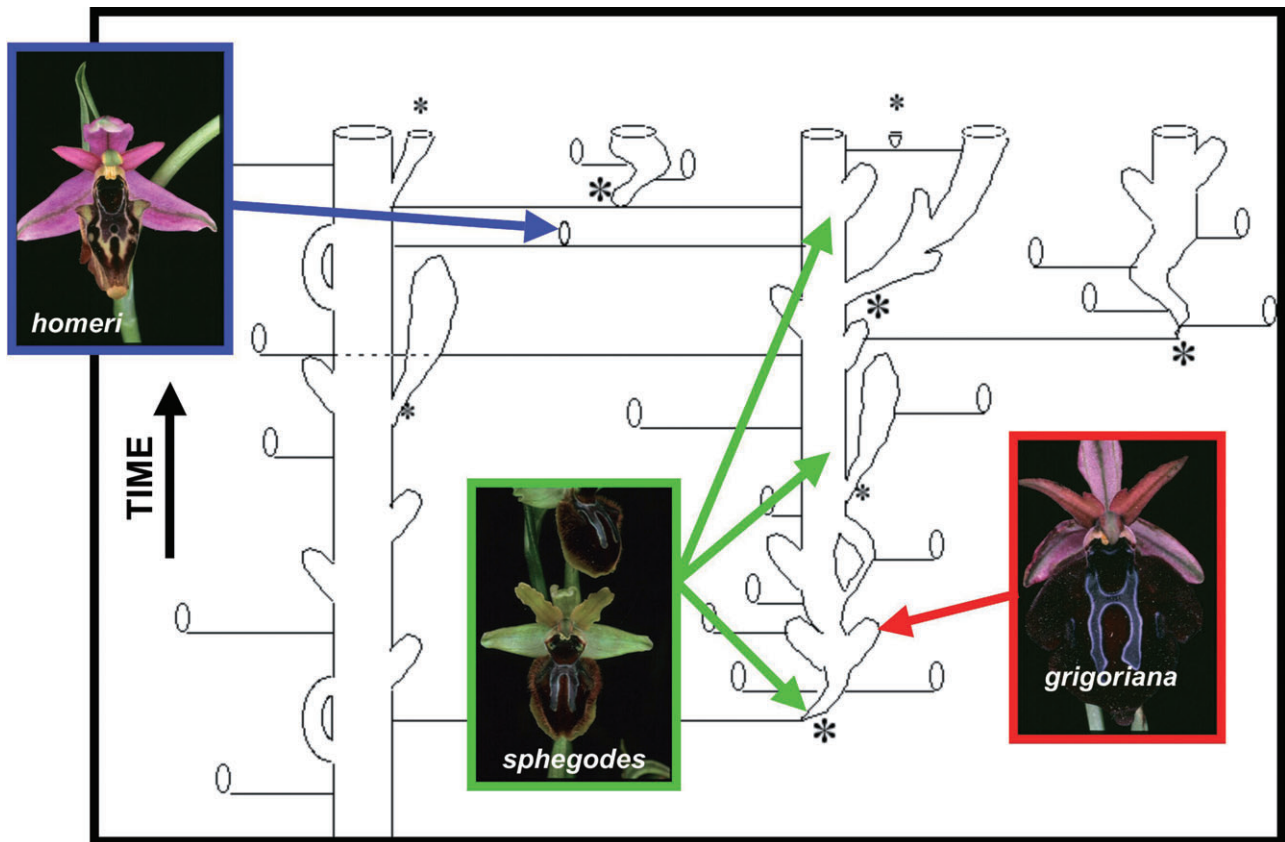


Figure 2. A theoretical phylogeny presented by Bateman & DiMichele (1994: fig. 8) as being representative of typical evolutionary patterns across the vascular land plants. The diagram distinguishes between a small number of geographically widespread, numerically abundant, long-lived ‘parental’ species that generate a large number of daughter lineages through either gradual ‘budding’, single-generation saltation or hybridization; it also emphasizes that most of the daughter lineages of each kind fail to achieve the levels of numerical abundance, longevity or distinctiveness that we believe are required for recognition as bona fide species. To be an accurate representation of evolution in *Ophrys*, this diagram would require many more ‘buds’ and transient hybrid populations. Within the genus, the ecologically successful long-lived species are rarely taxonomically controversial; it is the budded proiespecies and the hybrids that we would classify as, at best, infraspecific taxa, whereas Vereecken *et al.* (2011) and others choose to classify them as fully fledged species. Here, we have placed on the diagram ‘postage stamp’ images of selected taxa that we consider representative of bona fide species (*O. sphegodes*), infraspecific taxa that have ‘budded’ (*O. ‘grigorigiana’*), and transient hybrid swarms (*O. ‘homeri’*). Images: R. Bateman.

Vereecken *et al.* (2011) are justified in arguing that ‘*Ophrys*–pollinator interactions are flexible in time and space’ – that flexibility is expressed in rapid and constant turnover of transient entities that fail before they have achieved fixation of any meaningful characters, either phenotypic or genotypic. Our contention is that such proiespecies should be required to surpass basic thresholds of longevity, frequency and, most importantly, identifiability before they merit recognition as bona fide species. Specifically, we require a species to not only be monophyletic but also to possess ubiquitous character states that permit identification through both phenotypic (i.e. morphological taxonomy) and genotypic (i.e. DNA bar coding) methods

(Bateman *et al.*, 2010). In our experience, the ten macrospecies of *Ophrys* possess all of these properties, whereas the multitudinous microspecies possess none.

Many observers would no doubt rush to the defence of at least some of the *Ophrys* microspecies, arguing that they are in fact distinguishable phenotypically if not genotypically, and pointing to the obvious differences (primarily in size) between the putatively ancestral *O. sphegodes* and descendant *O. ‘garganica’* illustrated by us in Figure 2. But this would be to ignore the fact that, by selectively illustrating individuals from populations that we have sampled across Europe, we could easily produce a continuum of form

to connect *O. sphegodes* with *O. 'garganica'*. We might even achieve this goal by raising to adulthood the progeny of a single self-pollinated capsule (Fig. 1). Neither the morphological nor the molecular discontinuities that should separate bona fide species (cf. Bateman, 2001) can be discerned among *Ophrys* microspecies; provided that the sampling of populations is sufficiently dense and extends across the full geographical range of the macrospecies in question, phenotypic continua consistently emerge.

The main problem with the taxonomic approach adopted by both the ethologists and some traditional taxonomists is that extrinsic properties of the organisms are, in practice, being prioritized over intrinsic properties. Within a macrospecies such as *O. fusca*, each readily circumscribed geographical unit (e.g. Mediterranean island, mountain range) has, by definition, its own endemic species. Within that geographical unit, each differentiable habitat might be considered to maintain its own microspecies. If two individuals within that habitat are observed to have pollinia removed by different species of pollinator, those two individuals are interpreted as representing different sympatric microspecies. Such an approach reverses more credible taxonomic protocols, which initially consider intrinsic properties – first phenotype and then genotype – across the geographical and ecological range of the putative species and compare them with those of all other similar species. Only once the appropriate discontinuities separating such intrinsically circumscribed entities have been firmly established should attempts be made to determine extrinsic properties of those taxa, such as pollinator relationships, habitat preference and geographical distribution.

The negative consequences of prioritizing extrinsic over intrinsic properties are graphically illustrated by Mifsud (2011): 'Previously this endemic species to the Maltese islands was called *O[phrys] sphegodes* subsp. *melitensis* but was recently put in its own taxon of *O. melitensis* by J. Devillers-Terschuren and P. Devillers in 1992. For long it was included in the *O. lunulata* group B17, which the general appearance of its flowers suggests, but Hans-Erich Salkowski shortly showed that it is pollinated by *Chalicodoma sicula*. As all species of the *O. lunulata* group are pollinated by bees of the genus *Osmia* . . . and all those of the *O. bertolonii* group B18 by bees of the genus *Chalicodoma*, it seemed rational [sic] to include *O. melitensis* in the *O. bertolonii* group . . . the two genera of the corresponding pollinator bees giving clear delimitation of the two *Ophrys* groups.' Extending this argument to its 'logical' conclusion, presumably we should classify as conspecific all the flowering plants that are pollinated by honeybees, or perhaps all of those that are pollinated by the wind?

We are now left with only one burning question. Accepting that pseudo-pheromones have an important role to play in understanding the evolution of *Ophrys*, what role should they play in its taxonomy? Although the pseudo-pheromones are indeed an intrinsic property of *Ophrys* plants, they are the most ephemeral of all intrinsic properties. Unlike morphological and DNA characters, they cannot be obtained from herbarium specimens, including holotypes. In the field, morphology alone can be used at present, but we are confident that pocket sequencers will generate DNA sequences in the field within a few years (e.g. Bateman, 2011; Gutierrez, 2011). Whether these are needed in practice is questionable, given that the ten macrospecies of *Ophrys* are readily distinguishable using morphology alone. In contrast with the acquisition of DNA data, it seems unlikely that pocket mass spectrometers will soon be provided to allow routine field identification via quantitative analysis of pseudo-pheromone cocktails. In short, pseudo-pheromones are a useful taxonomic character in theory but not in practice. Only pollinator visits to plants rooted in natural habitats constitute a less useful 'taxonomic' character, given their great rarity (e.g. Paulus, 2006).

CONCLUSIONS

Vereecken *et al.* (2011) conclude that our view of the evolution and taxonomy of *Ophrys* 'misrepresents the current state of understanding of *Ophrys* ecology and evolution'; hence, they are 'determined to resist adopting the view that the genus *Ophrys* is exclusively limited to a relatively small number of 'macrospecies'. In addition, they announce a very clear (if somewhat subjective) aim in their future research: to 'work towards some sort of compromise relating to the number of species recognized, which probably lies somewhere between the extremes of 19 species recognized by Pedersen & Faurholdt (2007) and the 250+ species by Delforge (2005), among others'.

These statements imply that the differences between our own views and those of Vereecken *et al.* (2011) are relatively superficial and hence could be solved via 'horse trading' negotiations aimed at agreeing a compromise number of species in the genus. In contrast, we regard the difference between the two viewpoints as being fundamental to systematic biology. As detailed by Bateman *et al.* (2010), there is presently a profound conceptual disagreement regarding how one should define, circumscribe and identify a species – issues that still await an explicit protocol from the ethological school(s). We are therefore happy for the ethologists to continue 'resisting' our arguments, because persistence of at least two radically contrasting systematic viewpoints will offer the

primary consumers of taxonomic information (e.g. field biologists, ecologists, conservationists) a legitimate choice of classifications (cf. Bateman, 2011). Users can then decide for themselves whether the morphologically and genetically based circumscriptions that we and others have devised, or the ethologically and ecologically based circumscriptions advocated by Vereecken *et al.* (2011), constitute the more practical, reliable and usable entities.

In summary, our 'understanding of *Ophrys* ecology and evolution' [more accurately, 'evolutionary mechanisms'] is not at fault; rather, we are unwilling to allow such fragmentary and ethereal data sets to dictate classifications of the genus that are highly speculative, impractical and inconsistent with the better-founded taxonomic treatments of the remaining genera of European orchids.

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REFERENCES

- Arditti J, Ghani AKA. 2000.** Numerical and physical properties of orchid seeds and their biological implications. *New Phytologist* **145**: 367–421.
- Ascensão L, Francisco A, Cotrim H, Pais MS. 2005.** Comparative structure of the labellum in *Ophrys fusca* and *Ophrys lutea* (Orchidaceae). *American Journal of Botany* **92**: 1059–1067.
- Ayasse M. 2006.** Floral scent and pollinator attraction in sexually deceptive orchids. In: Dudareva N, Pichersky E, eds. *Biology of floral scent*. Boca Raton: CRC, 219–241.
- Bateman RM. 2001.** Evolution and classification of European orchids: insights from molecular and morphological characters. *Journal Europäischer Orchideen* **33**: 33–119.
- Bateman RM. 2006.** How many orchid species are currently native to the British Isles? In: Bailey JP, Ellis RG, eds. *Current taxonomic research on the British and European flora*. London: Botanical Society of the British Isles, 89–110. +plate.
- Bateman RM. 2009.** Evolutionary classification of European orchids: the crucial importance of maximising explicit evidence and minimising authoritarian speculation. *Journal Europäischer Orchideen* **41**: 501–572.
- Bateman RM. 2011.** The perils of addressing long-term challenges in a short-term world: making descriptive taxonomy predictive. In: Hodkinson TR, Jones MB, Waldren S, Parnell JAN, eds. *Climate change, ecology and systematics*. Systematics Association Special Volume 78. Cambridge: Cambridge University Press, 67–95.
- Bateman RM, DiMichele WA. 1994.** Saltational evolution of form in vascular plants: a neoGoldschmidtian synthesis. In: DS Ingram DS, Hudson A, eds. *Shape and form in plants and fungi*. Linnean Society Symposium Series 16. London: Academic Press, 63–102.
- Bateman RM, Crane PR, DiMichele WA, Kenrick P, Rowe NP, Speck T, Stein WE. 1998.** Early evolution of land plants: phylogeny, physiology, and ecology of the primary terrestrial radiation. *Annual Review of Ecology and Systematics* **29**: 263–292.
- Bateman RM, Hollingsworth PM, Preston J, Luo Y-B, Pridgeon AM, Chase MW. 2003.** Molecular phylogenetics and evolution of Orchidinae and selected Habenariinae (Orchidaceae). *Botanical Journal of the Linnean Society* **142**: 1–40.
- Bateman RM, Hollingsworth PM, Squirrell J, Hollingsworth ML. 2005.** Phylogenetics: neottieae. In: Pridgeon AM, Cribb PL, Chase MW, Rasmussen FN, eds. *Genera orchidacearum, 4. Epidendroideae 1*. Oxford: Oxford University Press, 487–495. + references.
- Bateman RM, Devey DS, Malmgren S, Bradshaw E, Rudall PJ. 2010.** Conflicting species concepts underlie perennial taxonomic controversies in *Ophrys*. *Cahors Société Française d'Orchidophilie* **7**: 87–101.
- Bradshaw E, Rudall PJ, Devey DS, Thomas MM, Glover BJ, Bateman RM. 2010.** Comparative labellum micromorphology in the sexually deceptive temperate orchid genus *Ophrys*: diverse epidermal cell types and multiple origins of structural colour. *Botanical Journal of the Linnean Society* **162**: 502–540.
- Delforge P. 2006.** *Orchids of Europe, North Africa and the Middle East*, 3rd edn. London: A & C Black.
- Devey DS, Bateman RM, Fay MF, Hawkins JA. 2008.** Friends or relatives? Phylogenetics and species delimitation in the controversial European orchid genus *Ophrys*. *Annals of Botany* **101**: 385–402.
- Devillers P, Devillers-Terschuren J. 1994.** Essai d'analyse systématique du genre *Ophrys*. *Naturalistes Belges* **75**: 273–400.
- Donoghue MJ. 2005.** Key innovation, convergence, and success: macroevolutionary lessons from plant phylogeny. *Paleobiology* **31**: 77–93.
- Glover BJ. 2007.** *Understanding flowers and flowering: an integrated approach*. Oxford: Oxford University Press.
- Gutierrez T. 2011.** Field identification of vectors and pathogens of military significance. In: Watson MF, Lyal C, eds. *Descriptive taxonomy serving biodiversity*. Cambridge: Cambridge University Press, Systematics Association special volume 80. In press.
- Hedrén M, Nordström S, Bateman RM. 2011.** Plastid and nuclear DNA marker data support the recognition of four tetraploid marsh-orchids (*Dactylorhiza majalis* s.l., Orchidaceae) in Britain and Ireland. *Biological Journal of the Linnean Society*. In press.

- Hollingsworth PM, Squirrell J, Hollingsworth ML, Richards AJ, Bateman RM. 2006.** Taxonomic complexity, conservation and recurrent origins of self-pollination in *Epipactis* (Orchidaceae). In: Bailey JP, Ellis RG, eds. *Current taxonomic research on the British and European flora*. London: Botanical Society of the British Isles, 27–44.
- Hunter JP. 1998.** Key innovations in the ecology of macroevolution. *Trends in Ecology and Evolution* **13**: 31–36.
- Javelle M, Vernoud V, Rogowsky PM, Ingram GC. 2011.** Epidermis: the formation and functions of a fundamental plant tissue. *New Phytologist* **189**: 17–39.
- King M. 1993.** *Species evolution: the role of chromosomal change*. Cambridge: Cambridge University Press.
- Kullenberg B. 1961.** Studies in *Ophrys* pollination. *Zoologiska Bidrag från Uppsala* **34**: 1–340.
- Leins P, Erbar C. 2010.** *Flower and fruit: morphology, ontogeny, phylogeny, function and ecology*. Stuttgart: Schweizerbart.
- Malmgren S. 2008.** Are there 25 or 250 *Ophrys* species? *Journal of the Hardy Orchid Society* **5**: 95–100.
- Mant J, Peakall R, Schiestl FP. 2005.** Does selection on floral odor promote differentiation among populations and species of the sexually deceptive orchid genus *Ophrys*? *Evolution* **59**: 1449–1463.
- Mifsud S. 2011.** MaltaWildPlants. Available at <http://www.maltawildplants.com> (accessed 12 January 2011).
- Molnár VA, ed. **2011.** *Atlas of Hungarian orchids*. Budapest: Kossuth [in Hungarian].
- Neiland MRM, Wilcock CC. 1998.** Fruit set, nectar reward, and rarity in the Orchidaceae. *American Journal Botany* **85**: 1657–1671.
- Noda K, Glover BJ, Linstead P, Martin C. 1994.** Flower colour intensity depends on specialized cell shape controlled by a Myb-related transcription factor. *Nature* **369**: 661–664.
- Noor M. 2011.** Current primary research projects in the Noor lab. Available at <http://www.biology.duke.edu/noorlab/projects.html#psper> (accessed 18 January 2011).
- Paulus HF. 2006.** Deceived males – pollination biology of the Mediterranean orchid genus *Ophrys*. *Journal Europäischer Orchideen* **38**: 303–353.
- Pedersen H, Faurholdt N. 2007.** *Ophrys: the bee orchids of Europe*. Kew: Kew Publishing.
- Pillon Y, Fay MF, Hedrén M, Bateman RM, Devey DS, van der Bank M, Shipunov AB, Chase MW. 2007.** Evolution and biogeography of European species complexes in *Dactylorhiza* (Orchidaceae). *Taxon* **56**: 1185–1208. + E1–E17.
- Raguso R. 2001.** Floral scent, olfaction and scent-driven foraging behaviour. In: Chittka L, Thomson JD, eds. *Cognitive ecology of pollination*. Cambridge: Cambridge University Press, 83–105.
- Ridley M. 1993.** *Evolution*. Oxford: Blackwell.
- Roy BA, Widmer A. 1999.** Floral mimicry: a fascinating yet poorly understood phenomenon. *Trends in Plant Sciences* **4**: 325–330.
- Rudall PJ. 2002.** Homologies of inferior ovaries and septal nectaries in monocotyleons. *International Journal of Plant Sciences* **163**: 261–276.
- Rudall PJ. 2007.** *Anatomy of flowering plants*, 3rd edn. Cambridge: Cambridge University Press.
- Rudall PJ, Bateman RM. 2002.** Roles of synorganisation, zygomorphy and heterotopy in floral evolution: the gynostemium and labellum of orchids and other lilioid monocots. *Biological Reviews* **77**: 403–441.
- Schiestl FP. 2005.** On the success of a swindle: pollination by deception in orchids. *Naturwissenschaften* **92**: 255–262.
- Schiestl FP, Cozzolino S. 2008.** Evolution of sexual mimicry in the orchid subtribe Orchidinae: the role of preadaptations in the attraction of male bees as pollinators. *BMC Evolutionary Biology* **8**: 27 (10 pp).
- Scopece G, Musacchio A, Widmer A, Cozzolino S. 2007.** Patterns of reproductive isolation in Mediterranean deceptive orchids. *Evolution* **61**: 2623–2642.
- Stöckl J, Schlüter PM, Steussy TF, Paulus HF, Fraberger R, Erdmann D, Schulz C, Francke W, Assum G, Ayasse M. 2009.** Speciation in sexually deceptive orchids: pollinator-driven selection maintains discrete odour phenotypes in hybridizing species. *Biological Journal of the Linnean Society* **98**: 439–451.
- Thomas K, Kolle M, Whitney H, Glover BJ, Steiner U. 2010.** Function of blue iridescence in tropical understory plants. *Journal of the Royal Society Interface* **7**: 1699–1707.
- Vereecken NJ. 2009.** Deceptive behavior in plants I. Pollination by sexual deception in orchids: a host–parasite perspective. In: Baluska F, ed. *Plant–environment interactions: from sensory plant biology to active behavior*. Berlin: Springer Verlag, 203–222.
- Vereecken NJ, McNeil JN. 2010.** Cheaters and liars: chemical mimicry at its finest. *Canadian Journal of Zoology* **88**: 725–750.
- Vereecken NJ, Schiestl FP. 2009.** On the roles of color and scent in a specialized floral mimicry system. *Annals of Botany* **104**: 1077–1084.
- Vereecken NJ, Streinzer M, Ayasse M, Spaethe J, Paulus HF, Stöckl J, Cortis P, Schiestl FP. 2011.** Integrating past and present studies on *Ophrys* pollination – a reply to Bradshaw *et al.* *Botanical Journal of the Linnean Society* **165**: 329–335.
- Whitney HM, Kolle M, Andrew P, Chittka L, Steiner U, Glover BJ. 2009.** Floral iridescence, produced by diffractive optics, acts as a cue for animal pollinators. *Science* **323**: 130–133.