Conflicting species concepts underlie perennial taxonomic controversies in *Ophrys*

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Abstract – Radically conflicting estimates of the number of species encompassed by a single genus often reflect paucity of taxonomic knowledge. However, intensive studies of the European terrestrial orchid genus *Ophrys* have led to contrasting monographs that recognise few species (19 species plus 65 subspecies: Pedersen & Faurholdt, 2007) or many species (251 species forming 32 complexes: Delforge, 2006), a contrast that understandably perplexes conservation bodies. The striking yet largely continuous variation in floral morphology of the flowers may have tempted some systematists to partition variation unusually finely. However, the main driver encouraging recognition of new species (the species 'discovery' rate is currently approximately ten per annum) is the assumption that each subtly distinct morphological variant in each geographically definable region has its own specific pollinating insect; a consequence of the pseudo-copulatory pollination mechanism that explains the three-dimensionality, heterogeneous colours and textures, and complex pseudo-pheromones of the remarkable *Ophrys* labellum. Our thoroughly sampled molecular phylogeny resolves only ten groups within the genus, five of which contain only one widespread species. The inferred relationships have prompted further research, including a comparative micromorphological study of the labellar epidermis. Although low sequence divergence would be expected in a recent phenotypic radiation, the maintenance within many individuals of multiple plastid haplotypes and multiple ITS alleles suggests much recent introgression among these supposedly reproductively isolated species. Together with breeding experiments that recover multiple 'species' from a single artificially self-pollinated flower, these data refute the popular species concept based on the hypothesis of one pollinator per trivial variant, which is, we believe, too constrained a system to allow further diversification. Frequent phenotypic convergence is evident, especially between genetically distinct western and eastern Mediterranean provinces separated by contrasting prevailing winds. Given this new evidence, we view most named phenotypes ('microspecies') as either infraspecific taxa or hybrid swarms.

Résumé – Les concepts taxonomiques d'espèces chez les *Ophrys* révélateurs de controverses conflictuelles récurrentes. La faiblesse de la connaissance taxonomique de certains genres est à l'origine d'estimations contrastées du nombre de leurs espèces. Ainsi, des études approfondies du genre *Ophrys,* un genre d'orchidées terrestres d'Europe ont abouti à la publication de travaux allant de la reconnaissance d'un nombre réduit d'espèces, 19 avec 65 sous-espèces par Pedersen et Faurholdt (2007), jusqu'à un nombre élevé, 251 espèces formant 32 complexes (Delforge, 2006), rendant perplexe les organismes de protection. L'impressionnante quoique largement continue variabilité de la morphologie florale a tenté certains systématiciens de distinguer des espèces de manière inhabituellement fine. Toutefois, la raison principale de la description de nouvelles espèces, au rythme d'une dizaine par an, est

basée sur l'hypothèse que chaque population liée à une région donnée identifiée par toute subtile modification morphologique avait son propre insecte pollinisateur. Ce mécanisme de pollinisation par pseudocopulation serait à l'origine de la complexité du remarquable labelle par son relief, ses couleurs et ses textures hétérogènes et les phéromones qu'il produit. Notre analyse du genre par phylogénie moléculaire, avec un échantillonnage rigoureux, révèle 10 groupes dont 5 ne comportent qu'une seule espèce de large répartition. Leur relation supposée a été à l'origine d'études supplémentaires dont celle présentée sur la micromorphologie de l'épiderme du labelle. Alors qu'une faible divergence de séquence était attendue dans un contexte de radiation rapide, la présence dans beaucoup d'individus de plusieurs haplotypes plastidiques ainsi que de multiples allèles ITS suggère la présence d'introgression entre ces espèces supposées isolées du point de vue reproductif. Ceci, allié avec les expérimentations de croisements qui ont permis de produire plusieurs « espèces » à partir d'une fleur autofécondée, réfute le concept populaire d'espèce basé sur l'hypothèse d'un pollinisateur spécifique par variant qui serait d'ailleurs aussi trop contraignant pour permettre une nouvelle diversification. Il y a évidence de convergences phénotypes fréquentes, particulièrement entre les régions « génétiquement » différentes de l'Est et de l'Ouest de la Méditerranée qui sont isolées par des vents dominants. En raison de ces nouvelles évidences, nous considérons que la plupart des phénotypes décrits ne sont que des taxons intraspécifiques ou des nébuleuses hybrides.

INTRODUCTION

Bee orchids of the charismatic genus *Ophrys* have been the subject of several taxonomic treatments, each based primarily on classical techniques that focus on the appearance of the species (e.g. Devillers & Devillers-Terschuren, 1994) or, more recently, their chromosomal patterns (D'Emerico *et al*., 2005). These studies have differed substantially in the number of species recognised. The most influential of the recent European orchid floras (Delforge, 2006) recognised 251 species but, a year later, a monographic study of the genus by Pedersen & Faurholdt (2007), essentially based on the same body of morphological data, recognised only 19 species, 65 subspecies and five stabilised hybrid aggregates. To add insult to injury, subsequent DNA-based studies of the bee orchids (Devey *et al*., 2008, 2009) could detect at best only ten groups that merit recognition as genetically defined rather than morphologically defined species. How on Earth can such wide disparity of perceived species numbers occur in such a well-studied orchid genus?

This disagreement regarding bee orchid diversity represents a particularly extreme example of a phenomenon that frequently afflicts taxonomy – a dichotomy between researchers who divide natural variation into as many units as possible (splitters) and others who aggregate those subtly different units into entities that they consider to be either more easily recognised or more biologically meaningful (lumpers). However, it is our contention the genus *Ophrys* has generated a third category of co-evolutionary taxonomists, the 'ultrasplitters'.

Arguably the most characteristic and iconic of all Mediterranean orchids (Figure 1), *Ophrys* is readily distinguished from all other genera using either the appearance of its flowers or the sequence of bases in its DNA; both are unique and instantly recognisable. Its remarkable and much-discussed flowers are fine-tuned to deceive naïve male insects into attempting to mate with its flowers, a process termed pseudo-copulation (Figure 2) (e.g. Paulus & Gack, 1990; Paulus, 2006). Not surprisingly, the bee orchids have become enshrined in the botanical literature as an archetypal example of adaptation in flowers to best fit specific pollinating insects (e.g. Cozzolino & Scopece, 2008; Schlüter & Schiestl, 2008). But although the genus is easily identifiable, many of its constituent species are not; attempting to separate putative

species within this genus can provoke at best frustration and at worst temporary insanity in even the most experienced field orchidologist.

Figure 1. *Ophrys speculum*, featuring a remarkable mirror-like labellum. (Photo: Richard Bateman)
Figure 1. *Ophrys speculum*, au remarquable "miroir" du labelle.

Figure 2. Male *Argogorytes* digger-wasp attempting to mate with the Fly Orchid, *Ophrys insectifera*. (Photo: Barry Tattersall)
Figure 2. Mâle de guêpe-fouisseuse d'*Argogorytes* tentant de s'accoupler avec une fleur d'Ophrys mouche, *Ophrys insectifera.*

Species are fundamental units

The species is the most fundamental taxonomic rank. All users of biological information and assessors of biodiversity employ the species as their basic unit of currency. But just as in finance there are many international currencies whose perceived relative values vary through time, so the various species concepts that have been proposed over the centuries (at least 26 were recognised in a frequently cited review by Mayden, 1997) fluctuate in their relative popularity. And just as attempts have been made to simplify the world economy by reducing the total number of currencies (witness the many consequences of the advent of the Euro), all biologists would agree that maintaining two dozen species concepts is counter-productive. Many would say that an ideal world would support only one category of species – a concept so powerful and predictive, yet so flexible and functional, that it would be accepted and used routinely by all biologists. In contrast, we would argue that achieving such a state of taxonomic nirvana is not only impossible in the present circumstances but is also undesirable, as the conflicts over species circumscription reflect a deeper set of important but inadequately resolved issues – issues that can make exploring the evolutionary relationships among orchids especially exciting.

Three key criteria

We believe that Mayden's two dozen species concepts can legitimately be reduced to just three primary criteria for circumscribing species. The first, and most traditional, concept is simply similarity of appearance – morphology. This rather nebulous concept has underpinned orchid taxonomy for the last three centuries, and continues to do so. Basically, we expect every

individual within a species to possess one or more characters (or combinations of characters) that reliably separate it from individuals of all other species – such characters are said to be diagnostic and fixed (for example, the reflective speculum in the centre of the labellum that characterises the genus *Ophrys*: e.g. Figure 1). Of course, we have to sort through a lot of characters – and preferably a lot of individual plants from a lot of populations – before we can be confident that we have identified the most reliable diagnostic characters.

The second criterion, which was presaged by Darwin but took firm hold only in the middle of the 20th Century, is reproductive isolation. The foundation of what soon became known, rather arrogantly, as **the** biological species concept, this principle attempted to encompass the evolutionary process that was considered most likely to have given rise to the resulting species. Population geneticists and reproductive biologists took Darwin and Wallace's concept of natural selection – competition for resources within populations leading to a set of individuals that are on average better adapted to their immediate environment – and couched it in terms of ecological interactions within populations. If the individuals were capable of interbreeding within natural populations without incurring serious damage to their fitness (i.e. their ability to both survive and reproduce in that environment) they were considered to belong to the same species. The evidence of their interbreeding could be ecological – for example, observing animals transferring pollinia between orchids – or it could be genetic – for example, using genetic fingerprints to infer the relationships between individuals and to determine whether, and how often, those individuals exchanged genes (a process termed gene flow). In an ideal world, the absence of gene flow would be sufficient to circumscribe an orchid species, just as it helps to distinguish humans from chimpanzees and gorillas.

The third and most recently devised criterion, perhaps the most difficult to explain, is termed monophyly. A monophyletic group can be defined (somewhat simplistically) as encompassing all of the descendants of a single presumed ancestor. The relationships among the organisms analysed are inferred by constructing evolutionary trees, using morphological or genetic information, and partitioning the tree into monophyletic taxa by cutting particular branches. This approach has the huge advantage of ensuring that the circumscribed group is 'natural' – both self-defining and the product of evolutionary change (e.g. Bateman, 2007, 2009b). However, there remains a crucial ambiguity; is the basic unit of comparison – the ancestor and descendant(s) – the gene, the individual organism, the local populations, the meta-populations or the species? In practice, the principle of monophyly has justly become fundamental to rearranging all species in order to circumscribe only natural genera, but it is far less clear whether monophyly is useful at a lower level, for aggregating individuals and/or populations into species. The main problem is that it relies on repeated events that separate and thus isolate populations, but it cannot readily accommodate circumstances in which these daughter populations recombine to once again form a single interbreeding population, a process that occurs commonly in the real world.

Returning to Mayden's (1997) two dozen species concepts, most are in practice generated by combining one, two or even all three of the above principles into a single definition; for example, Scopece *et al*. (subm.) have attempted to combine monophyly and reproductive isolation in order to test the validity of competing classifications of subtribe Orchidinae. For the rest of this article, we have chosen to focus on the relationship between similarity of morphological appearance and reliability of reproductive isolation, because we believe that it is this uneasy relationship between radically different ways of studying species that lies at the crux of the great *Ophrys* controversy. It also helps to explain why everyone, from the casual novice to the specialist researcher, experiences serious difficulties when attempting to identify bee orchid species in the field.

Identification requires circumscription

We can all assign an unknown plant to the genus *Ophrys* on overall appearance (jizz) alone, without needing to memorise a range of reputedly diagnostic characters. If we dissect the bee orchids' insect-mimicking jizz into particular characters, we might begin by noting the unusually large size difference between the three sepals and the two much smaller lateral petals. However, it is more likely that we would be drawn immediately and inexorably to the spectacular and complex labellum (Figure 1). The facts that it is (a) three-lobed with a notched central lobe and (b) folded three-dimensionally fail to distinguish it from many other orchid genera. However, more remarkable is the fact that its folding is convex rather than concave (thus preventing the labellum from forming a nectar-secreting spur – spurs are the ultimate expression of concavity), bears at least some well-developed hairs (trichomes) along the margin (Figure 3), and has at its centre a smooth, lobed, differently coloured, more reflective

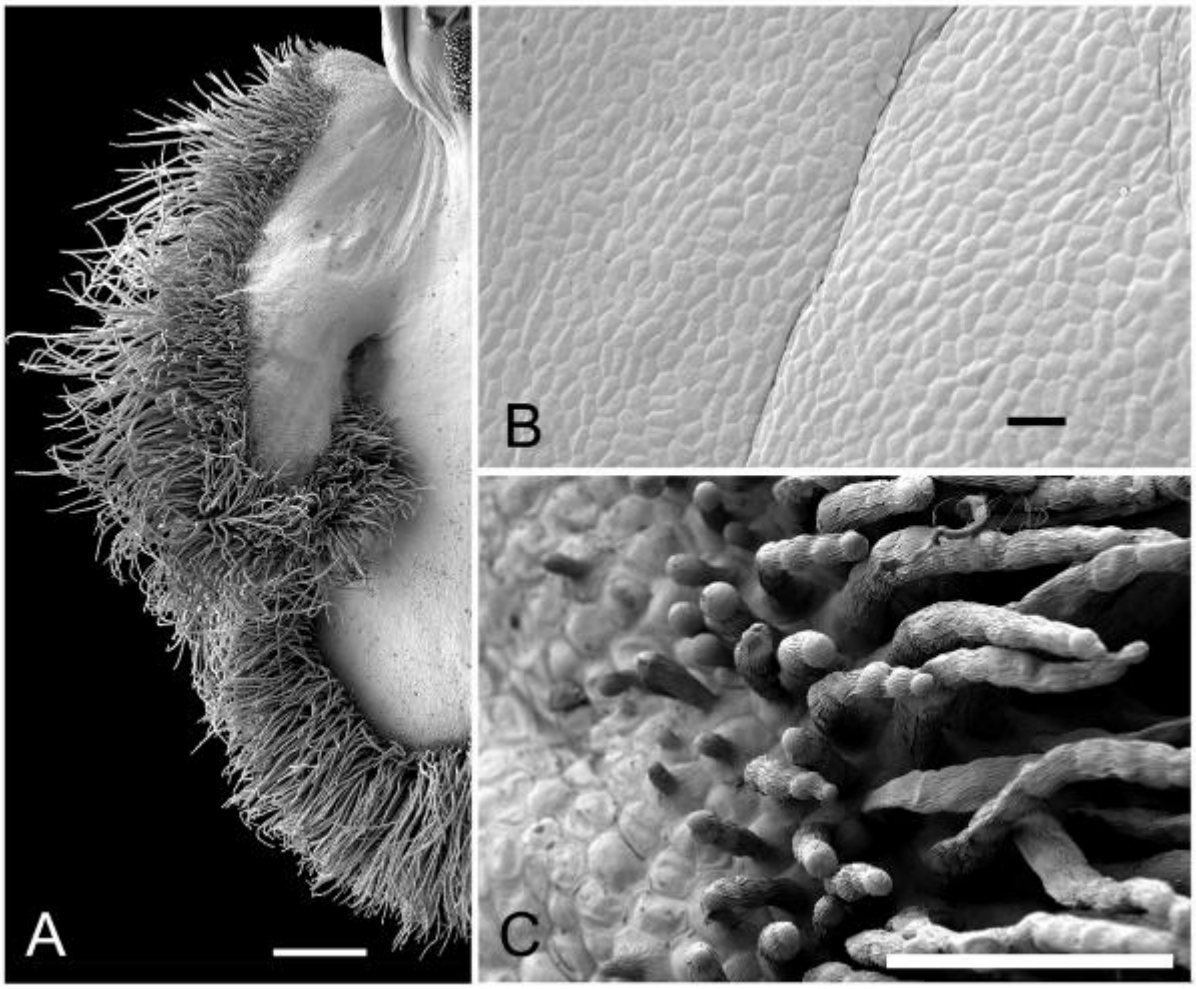

Figure 3. Scanning electron microscope plate of the labellum of *Ophrys speculum*, showing the remarkable variety of surface features that serve to attract pollinating insects. A, left half of labellum; B, enlargement of speculum; C, enlargement of marginal hairs. Scales: A = 1 mm, B and C = 0.1 mm. (Photos: Beth Bradshaw)

Figure 3. Images en microscopie électronique à balayage du labelle d'*Ophrys speculum*, montrant la remarquable diversité des caractères de surface servant à attirer les insectes pollinisateurs. A. moitié gauche du labelle ; B. agrandissement du speculum ; C. agrandissement des poils des marges. Echelle : A = 1 mm, B et C = 0.1 mm

and in some cases iridescent feature termed the speculum (e.g. Figure 1). It is this basic knowledge, understood either explicitly or more often implicitly, that allows us to readily assign a particular plant to the genus *Ophrys*.

But we are not concerned here with merely identifying a plant as an *Ophrys* – anyone can do that. We are far more ambitious – we wish to know which of Delforge's (2006) 251 finely divided *Ophrys* species – perhaps better henceforth described as microspecies – lies at our feet (potentially a serious challenge, bearing in mind that an unusually rich Mediterranean site could offer as many as a dozen Delforgean *Ophrys* species). Pinning down a population to a Delforgean microspecies can become a serious plant-by-plant challenge – a challenge that often results in the tentative conclusion that two or more morphologically similar species are somehow co-existing at the locality in question. The descriptions and identification keys in monographs and floras are often insufficiently precise and detailed (or, conversely, insufficiently understandable) to distinguish among such closely similar species. We should consider the possibility that these failures of identification are not the fault of the technical guides or ourselves, but rather that we are attempting to identify species that do not actually exist.

Morphology *versus* morphology

Having established the background to the bee orchid debate, we can now return to the conflicting results of the three studies that began this article. The monographs of Delforge (2006) and Pedersen & Faurholdt (2007) have much in common. Both books are the work of highly experienced orchidologists who have accumulated a tremendous amount of field experience; these authors know bee orchids not as desiccated grey-brown cadavers on herbarium sheets but as living, breathing plants carpeting Mediterranean hillsides. Both studies are traditional, in the sense of being based primarily on morphological data reflecting a wide geographical spread of observations (though Pedersen & Faurholdt surprisingly chose to ignore the bee orchids of Asia Minor and North Africa). Given the same basic information, how can Delforge possibly conclude that there are 251 species of *Ophrys* while Pedersen & Faurholdt opt for a mere 19 species? Part of the answer lies in contrasting treatments below the species level. Pedersen & Faurholdt formally recognise 65 subspecies and five taxonomically broad hybrid aggregates, whereas Delforge deliberately mimimises use of these lower ranks in his exceptionally egalitarian classification. Clearly, most of the names recognised as species by 'splitter' Delforge have either been treated as subspecies or hybrids or have been completely ignored by 'lumpers' Pedersen & Faurholdt. Perhaps these conflicted authors are using different definitions of a species?

For Pedersen & Faurholdt (2007), "a species consists of all individuals that in natural conditions (in reality or potentially) can interbreed to produce consistently viable and fully fertile offspring; … individuals of one species are distinguished from those of other species by morphological features." Delforge (2006) elected to list the defects inherent in the various species concepts available to him but refused to offer a formal definition of a species other than to state that his approach is fundamentally phylogenetic (i.e. is intended to emphasise 'natural' monophyletic groups). He did, however, make two especially relevant observations. Firstly, "the number of defined species depends on the level of [morphological] differentiation used by the taxonomist. The level is arbitrary. If a high threshold of differentiation is applied there will be few species with large interspecific variation [=lumping]; using a low threshold there will be many species, with all visibly different taxa becoming species [=splitting]." Is this distinction sufficient to explain these radically different treatments offered by Delforge and Pedersen & Faurholdt? And is it truly arbitrary?

We should also consider the second pertinent statement made by Delforge. "Another pitfall awaiting the taxonomist is the application of tools [including DNA-based analyses] that

allow analyses and taxonomic decision-making … Each specialist has a tendency to give weight to the results of his own technique, sometimes to the point where they discredit the study of morphological characters, and arrive at a classification based on a single technique; this is best avoided." At first glance, this statement appears to be an effective defence of traditional morphology. But what is traditional morphology if not a "single technique"? And just what **do** we learn when other kinds of information, such as DNA sequences, are considered?

Morphology *versus* molecules

The answer to the last question came when, building on more taxonomically and genetically restricted molecular analyses (Bateman *et al*. 2003; Soliva & Widmer 2003; Schlüter *et al*. 2007), Devey *et al*. (2008) sampled all of the major groups of bee orchids species recognised on traditional morphological grounds by Delforge (2006) and Pedersen & Faurholdt (2007) and gathered no less than four kinds of data. They approached morphology by measuring 45 different features of plants sampled widely from populations distributed across Europe. They then accumulated data on the sequence of nucleotides (a language of only four letters: A, G, C and T) from two discrete DNA-based genomes: the chromosomes of the nucleus, which are inherited equally from the mother and father, and the plastids (green, chlorophyll-rich bodies within cells, termed organelles), which are inherited only from the mother. Because these methods analyse only a tiny portion of the total DNA, Devey *et al*. (2008) also used a more inclusive technique termed AFLP, which cuts all of the chromosomes into fragments and then measures the length of each resulting fragment.

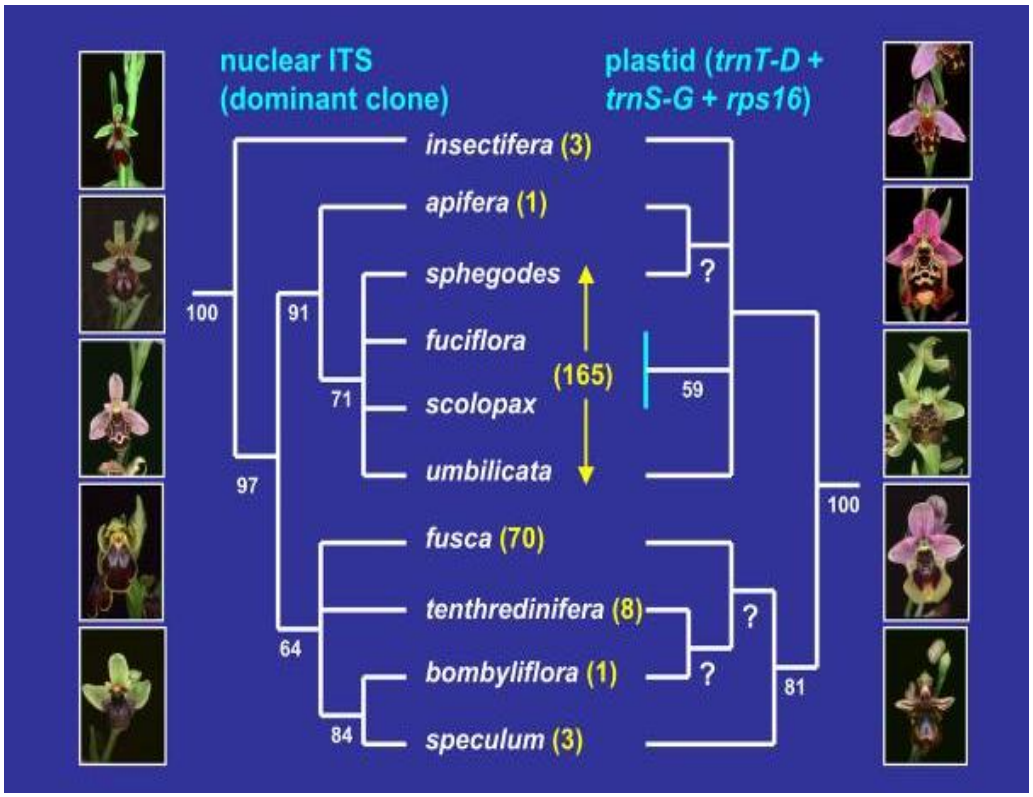

Figure 4. Simplified evolutionary trees of major groups of *Ophrys* based on chromosomal (left) and plastid (right) DNA, surrounded by representatives of the ten major groups thus circumscribed. (Image: Richard Bateman)

Figure 4. Arbres évolutifs simplifies des principaux groupes d'*Ophrys* bases sur l'ADN chromosomique (à gauche) et des plastides (à droite), liés à des représentants des 10 groupes majeurs ainsi identifiés.

The plastid analyses yielded the same number of distinct groups of supposed species, namely nine, epitomised by the following familiar species: *O. insectifera*, *O. tenthredinifera*, *O. bombyliflora*, *O. speculum*, *O. fusca*, *O. apifera*, *O. umbilicata*, *O. fuciflora* and *O. sphegodes* (Figure 4). AFLP gave similar results but failed to discriminate clearly among *O. speculum*, *O. tenthredinifera* and *O. bombyliflora*. Chromosomal DNA (ITS sequences) were slightly more discriminatory, tentatively further dividing the *O. fuciflora* group into two subgroups based on *O. fuciflora s.s.* and *O. scolopax s.s.* and thus delimiting ten groups of *Ophrys* altogether. So three radically different kinds of genetic analysis are telling essentially the same story; the genus contains only nine or, at most, ten units that are demonstrably genetically distinct and so are unequivocally reproductively isolated. These techniques also simultaneously allow us to determine whether these groups are monophyletic (inclusive, self-defining groups with a single evolutionary origin). If, as some theorists have argued, reproductive isolation and/or genetically determined monophyly should be used to delimit species, there are clearly nine or ten species of *Ophrys* – not even the 19 species recognised by Pedersen & Faurholdt, and certainly not the 251 species recognised by Delforge.

Encouragingly, when found in the field, these ten 'macrospecies' can readily be distinguished from each other by any competent botanist. Rather, it is when attempts are made to further fragment these species into much more subtly distinct 'microspecies' that even experts experience great difficulty in distinguishing among them. Indeed, the DNA data tell us that very similar floral morphologies can be found in different genetic groups; for example, prominent lateral 'horns' on the labellum have evolved independently in the *scolopax* (Figure 5) and *fuciflora* (Figure 6) groups, and similar labellum shapes and markings have evolved independently in the *umbilicata* (Figure 7) and *sphegodes* (Figure 8) groups (Devey *et al.*, 2008, 2009), undermining some relationships previously inferred from morphological phylogenetic analysis (cf. Devillers & Devillers-Terschuren, 1994). In contrast, comparative micromorphological studies of the exceptionally diverse epidermal cells of the labellum (the

Figures 5, 6. Two microspecies that have independently evolved labellar horns: *Ophrys scolopax s.s.* (*scolopax*) from S France (left) and *O. lapethica* (*umbilicata*) from Chios (right). (Photos: Richard Bateman)

Figures 5, 6. Deux micro-espèces dont les cornes du labelle ont évolué indépendamment : *Ophrys scolopax s.s.* (*scolopax* du sud de la France) (à gauche) et *O. lapethica* (*umbilicata*) de Chios (droite).

Figures 7, 8. Two microspecies that have independently evolved similar labellum shapes and markings: *Ophrys kotschyi* (*umbilicata*) from Cyprus (left) and *O. cretica* (*sphegodes*) from Crete (right). (Photos: Richard Bateman)
Figures 7, 8. Deux micro-espèces dont la forme et les marques du labelle ont évolué de manière : *Ophrys kotschyi* (*umbilicata*) de Chypre (à gauche) et *O. cretica* (*sphegodes*) de Crète (à droite).

most critical portion of the flower for inducing pseudo-copulation) have supported the novel relationships, for example providing evidence of a close relationship between the *tenthredinifera*, *bombyliflora* and *speculum* groups (Bradshaw *et al*., 2010). And we have shown that, even if those fieldworkers had in their pockets an automated DNA analyser (technology that should become widely available within the next few years), they would still be unable to differentiate among the highly divided microspecies. More broadly, if all three of our species criteria – morphological similarity, monophyly and reproductive isolation – are telling us the same story, why is there any remaining controversy? How can one arch-splitter still write that "genetic analyses have brought no insight to speciation studies"? Surely the lumper's case that genetically delimited macrospecies are preferable to co-evolutionarily delimited microspecies is proven beyond all reasonable doubt?

The elephant in the room?

In fact, there is one valid counter-argument that the splitters could have chosen to deploy in defence of their position. By definition, the genetic techniques employed by Devey *et al*. (2008) and others circumscribe groups that have very little if any gene flow between them; the groups of orchids that are recognised undoubtedly pass the reproductive isolation test. But there would have been a period (probably only a brief period) when these species were reproductively isolated but would have been unlikely to be detected by the genetic methods employed by Devey *et al*. (2008). Reproductive isolation develops when one or at most very few genetic changes occur, spread through the population in question and eventually characterise every plant in the population – they are then said to be fixed (Figure 9). And yet more generations must pass before enough additional genetic differences accrue to readily differentiate this new species from all other species – not least from its ancestral species. In other words, if reproductive isolation precedes substantial genetic divergence of

species by many generations (a period termed the 'genetic divergence lag' by Bateman, 2009b), 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. Here, perhaps, is the elephant in the room …?

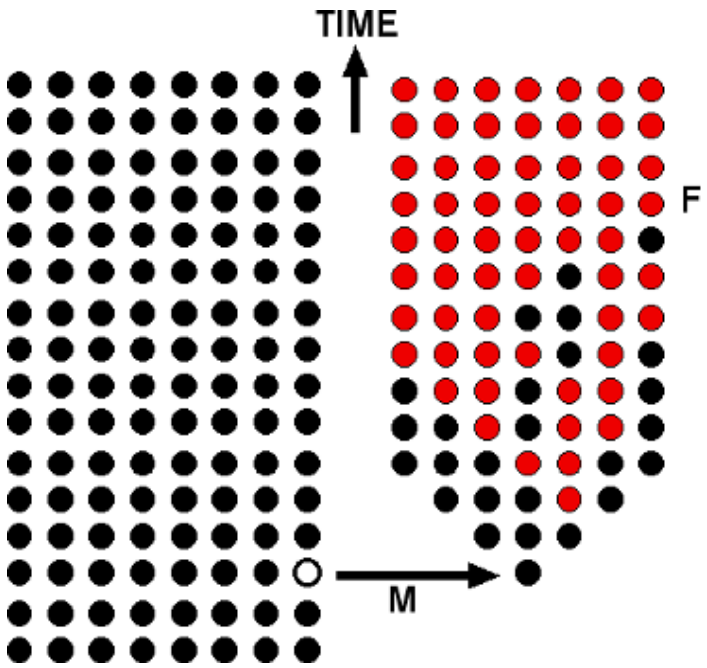

Figure 9. Diagram showing the hypothetical fates of an ancestral and daughter population which first become physically isolated due to migration (M) of the daughter population (e.g. from the mainland to an island) and several generations later become reproductively isolated due to the fixation (F) of a novel feature that arose within the daughter population.

Figure 9. Diagramme montrant la situation hypothétique de populations mères et filles devenues physiquement isolées suite à une migration (M) de la population fille (par exemple d'un continent vers une île) et qui est devenue génétiquement isolée au bout de quelques générations en raison de la fixation (F) d'un caractère nouveau apparu dans la population fille.

Actually, it may be more accurate to refer to 'the fly in the ointment' than 'the elephant in the room', because other biologists have explored reproductive isolation in *Ophrys* not through indirect genetic assessment of gene flow but rather through direct observation of their pollination mechanisms. And what magnificent pollination mechanisms they are! The bee orchid flower is beautifully adapted for an uncommon pollination strategy termed pseudo-copulation. Specifically, inexperienced male insects, mostly bees and wasps, are sufficiently attracted to the *Ophrys* flower – first by smell (a sophisticated cocktail of chemicals that mimic the sexually attractive pheromone chemistry of the female bee or wasp), then by appearance (the kaleidoscopic colours and iridescent speculum of the labellum) and finally by touch (the contrasting hairs on different regions of the labellum) – into attempting to mate with the flowers. In doing so they accumulate pollen masses and, having failed to learn their lesson, they then transfer the pollen masses to another flower when they make a further abortive attempt to mate with an orchid (e.g. Paulus & Gack, 2000; Paulus, 2006; Schiestl & Cozzolino, 2008; Schlüter & Schiestl, 2008). The insect gains little from this co-evolutionary 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! Not surprisingly, the bee orchids have become a much publicised cause célèbre – a model system in the realm of adaptive evolution.

The problems really begin when we retool this incontrovertible evolutionary mechanism for use (or abuse?) as a key taxonomic character. Evolutionary theory tells us that *Ophrys* flowers have become co-adapted to dupe the sexually frustrated males of one particular species of insect. So if a particular species of insect is observed to pollinate a particular species of *Ophrys*, it is tempting to assume that evolution has operated with such precision that each pollinating insect has acquired a faithful co-evolutionary relationship with a

different *Ophrys* species. In effect, the identity of the pollinator becomes the diagnostic character of the *Ophrys* plant. This in turn means that observing new pollinators *in flagrante delicto* leads to a proliferation of new orchid species descriptions (e.g. Paulus & Gack, 1990; Paulus, 2006; Schlüter *et al*., 2007).

Of course, these authors also seek morphological characters that might separate the new (and typically very localised) orchid from the more widespread species to which it was previously attributed, but any prospective diagnostic characters found are inevitably few and disconcertingly subtle, and are usually derived from studying few if any populations in detail. And as we have already clearly seen, DNA characters are unlikely to come to our rescue when we attempt to identify this novel species. Thus, pollinator observations are in practice being prioritised over genetic information when trying to infer whether reproductive isolation has been achieved by a particular group of orchids. This assumption has been further challenged by Bateman (2009a, b; also Bateman *et al*., in prep.), who argued from first principles that the inferred perfection of the one-to-one relationship between pollinator and orchid 'species' would strongly constrain any further diversification. A species possessing multiple pollinators – differing in importance from site to site, time of day to time of day, day to day and year to year – provides much greater opportunities for generating further recognisable local variants.

Insights from horticulture

Fortunately, there is a third approach available to study reproductive isolation. Instead of observing the interactions of orchids with pollinating insects in the wild, we can bring the orchids into cultivation, substitute a paintbrush for the insect when transferring pollen between flowers, and examine the appearance and fertility of the progeny of these controlled crosses. The results have been most illuminating.

Firstly, almost all crosses between species generate highly fertile offspring, but surprisingly, there was no correlation between degrees of genetic differentiation and degree of loss of fertility in hybrids (Scopece *et al*., 2007; Cozzolino & Scopece, 2008). Unfortunately, these studies only performed crosses between members of the ten *Ophrys* species recognised by Devey *et al*. (2008). Had the authors crossed microspecies within each of these ten groups, we would have predicted little or no substantial decrease in fertility. It has even been possible to combine the genes of as many as seven genetically contrasting species into a single artificial hybrid (Malmgren, 2008). Secondly, it has proven easy to cross two genetically distinct species that co-exist in the wild in order to (re)create other co-existing species that are recognised by splitters but are not actually genetically distinct (Malmgren, 2008). In other words, as correctly inferred by Pedersen & Faurholdt (2007), many of the *Ophrys* microspecies recognised by splitters such as Delforge (2006) are almost certainly recently formed hybrid populations rather than bona fide species. Provided that the parents are genetically distinct from each other, they can be reliably identified. Indeed, if genetic data are gathered from both the chromosomes (inherited from both parents) and the plastids (inherited from the mother only), we can even determine which species was mother and which was father (e.g. Soliva & Widmer, 2003; Bateman *et al*., 2008; Cortis *et al*., 2009).

Thirdly, and most strikingly, a single self-pollinated *Ophrys* capsule can generate a set of seeds that, when grown to maturity, shows a range of morphological diversity equal to three or more supposed microspecies (Malmgren, 2008). This constitutes an especially graphic illustration of why subtle morphological differences should not be viewed as marking species boundaries without independent genetic evidence that they have become reproductively isolated. Nor has morphological diversity been treated evenly across the genus by morphologists. For example, populations attributed to *Ophrys sphegodes sensu stricto* and *O. fuciflora sensu stricto* in southern England each contain as much morphological variation as

Mediterranean populations of *O. sphegodes sensu lato* and *O. fuciflora sensu lato* that each supposedly contain several microspecies.

CONCLUSIONS AND BROADER IMPLICATIONS

At best, the splitters' microspecies of *Ophrys* are not reliably identifiable by any known method. The morphological characters are too subtle, the molecular characters non-existent, and pollinator visits too rare, to assist identification. At worst, the entire ledger of bee orchid microspecies is an artifice of man, which has spawned a popular mythology of recent and rampant speciation. Because considerable variation through space and time is highly likely, an adequate test of the pollinator-centred view of species circumscription would require observations of pollination of any putative *Ophrys* species to be made across its entire geographical range, in all its habitats, throughout its flowering period, through many years. This is where the indirect nature of genetic analyses becomes a strength rather than a weakness, because the genetic markers reflect the reproductive behaviour of the species over a substantial period of time, rather than being subject to the vagaries of a particular ecological interaction in a particular place at a particular point in time (Scopece *et al*., subm.).

More pragmatically, most of the many assertions of pollinator fidelity that argue in favour of a one-to-one relationship between orchid and pollinator are discredited when sufficient observations are made; most orchids have more than one pollinator, and most pollinators service more than one orchid (Bateman *et al*., in prep.). To use supposed pollinator specificity as a taxonomic tool, in the absence of corresponding genetic data, is to allow the ecological tail to wag the genetic dog. We therefore hereby mischievously withdraw the lifeline that we offered to the splitters nine paragraphs previously. A few *Ophrys* species might currently reside in the brief interlude between achieving reproductive isolation and genetic distinction – perhaps enough to stretch from the 10 species of Devey *et al*. (2008) to the 19 species of Pedersen & Faurholdt (2007). For example, given their contrasting appearances, it is tempting to hypothesise that *O. bertolonii* (Figure 10) should be at least potentially reproductively isolated from *O. sphegodes* (Figure 11), despite the similarity of their DNAs.

Figures 10, 11. Two microspecies that are genetically similar but might be supposed on morphological grounds to have achieved reproductive isolation: *Ophrys drumana* in the *bertolonii* group (*sphegodes*, SE France, left) and *O. sphegodes s.s.* (UK, right). (Photos: Richard Bateman)

Figures 10, 11. Deux micro-espèces qui sont génétiquement similaires mis qui sont supposes être isolées reproductivement : *Ophrys drumana* du groupe *bertolonii* (*sphegodes*, SE France, à gauche) et *O. sphegodes s.s.* (UK, à droite).

However, to argue that 241 of Delforge's (2006) 251 *Ophrys* species simultaneously reside in this transient evolutionary nomansland stretches credulity well beyond breaking point. The great majority of the microspecies are, in short, 'Emperor's New Clothes' species, visible only to their human creators.

We might end by asking whether victory in this taxonomic debate could be anything other than pyrrhic. Isn't determining the number of *Ophrys* species in the Mediterranean worryingly similar to counting angels on the head of a pin – an unwelcome diversion from more pressing matters such as conserving these charismatic and greatly valued orchids? We think not. This is a debate of more than academic interest, as conservation priorities are likely to be strongly influenced by whether *Ophrys* is considered to exhibit rampant hybridisation and constitute between 2% (Devey *et al*., 2008) and 4% (Pedersen & Faurholdt, 2007) of the Mediterranean orchid flora, or to exhibit negligible, mostly anthropogenic hybridisation and to constitute about 50% (Delforge, 2006; Paulus, 2006) of the Mediterranean orchid flora. And when deciding conservation priorities, how does one balance the needs of a total of a few hundred individuals of a supposed endemic such as *O. homeri* on the Aegean island of Chios (Figure 12: most likely representing recently formed hybrid swarms between members of the *fuciflora s.l.* and *sphegodes s.l.* groups) against a less taxonomically equivocal and equally charismatic species residing in another genus? Surely they should not be weighted equally in conservation programmes?

Figure 12. *Ophrys homeri* (*fuciflora*), a rare putatively endemic microspecies from the Aegean island of Chios that is suspected to be of hybrid origin. (Photo: Richard Bateman)
Figure 12. *Ophrys homeri* (*fuciflora*), une espèce rare endémique putative de l'île de Chios, Mer Egée, qui est supposée d'origine hybride.

At this point, the arguments for and against recognising subspecies tend to become more political than scientific, reflecting innate prejudice against the rank of subspecies (Bateman, 2009a). For example, we learn that "infraspecific taxa rarely represent evolutionarily significant units that could be useful in conservation" (Pillon & Chase, 2007, p. 265). From our own perspective, potentially the main benefit of Delforge-style splitting is that lumpers such as ourselves can subsequently lump the contents of any splitter's inventory lists, whereas when faced with a lumper's inventory list, a splitter cannot by definition achieve the level of precision that he or she desires. The real problems begin when a splitter actually believes that his or her inventory is wholly biologically meaningful, and that the microspecies recorded are the equal in significance to any other plant species. The decision of Pedersen & Faurholdt to recognise many of Delforge's microspecies as either subspecies or hybrids has much to commend it, as we can still employ Delforgean microspecies names but at a lower and more appropriate taxonomic level, thereby acknowledging their weaker claim to conservation resources compared with other, bona fide species. As noted by Delforge, usage of subspecies rank has declined in recent years, but we would argue that this reflects more of a taxonomic fashion statement than the outcome of an informed scientific debate among the many interested parties.

Lastly, some more general lessons about science can usefully be taken from the *Ophrys* debate. It is best to avoid exaggerating the effectiveness of any one kind of data, but rather to seek to understand the constraints operating on each kind. Once this has been done, gather the

kinds of data that are most suitable for addressing the questions that you originally set out to answer, and analyse the data in a rigorous way that includes relevant and precise definitions of taxonomic ranks (Bateman, 2009b). Study the organisms in the field, as frequently and widely as is feasible. And above all, remain sceptical; we do not yet possess conclusive answers to any of the key questions concerning the evolution and classification of *Ophrys*, that most charismatic of European orchid genera.

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