More grist for the mill?: Species delimitation in the genomic era and its implications for conservation

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Abstract

Species delimitation is one of the most contested areas in modern biology, with widespread disagreement about almost every aspect of the definition and implementation of the "species" label. While this debate is intellectually stimulating, it also has real implications for conservation, where its impacts on taxonomic inflation or inertia can mean that specific populations receive adequate conservation measures or are ignored. Recently, the rise of next generation sequencing and phylogenomics has revolutionised phylogenetic understanding of many organismal groups but has simultaneously highlighted the porosity of genomes in terms of admixture across previously delineated species barriers. The extraordinary power of genomic data is increasingly being used to delineate species, and several publications in this domain have recently attracted significant attention and criticism. Here we revisit the question of species delimitation, but from a genomic context. We ask how and whether the large amounts of data provided by genomic methods can resolve the longstanding discussion on the validity and application of phylogenetic and allied species concepts, and how some recent examples can inform this debate. We argue that conserving adaptive potential is a priority for conservation, and no single species concept currently does that adequately on its own. Genomic data holds the potential to add unprecedented detail, but frequently falls short of this potential.

INFLATION OR INERTIA?

Due to the pivotal role of the species as the most important unit of biodiversity, conservation planning must be based on a good understanding of species number, diversity and endemism, measurements that only make sense within the context of consistent taxonomic classifications (Isaac, Mallet and Mace, 2004; Zachos *et al.,* 2013). However, as a result of the many different epistemological views on the

species concept (e.g. Avise and Ball, 1990; Wheeler and Platnick, 2000; Baker and Bradley, 2006), and due to the gradual process of evolutionary divergence, there is a continuum of genomic divergence patterns and estimates for which different researchers would consider speciation to be 'complete' (DeQueiroz, 1998). Some evolutionary biologists have classified populations as the same species unless strong evidence to the contrary exists, *e.g.* reproductive incompatibility or reciprocal monophyly (with the archaic term 'lumpers'; Heller *et al*., 2013). The genealogical concordance method of phylogenetic species recognition (often known as the genealogical species concept, or GSC; Avise and Ball, 1990; Baum and Shaw, 1995), as well as the Biological species concept (BSC), often result in a high threshold of species recognition. The GSC usually considers two populations to be designated species only when they are "*isolated long enough [that]* ***all*** *gene-genealogies will be concordant*" (emphasis ours; Baum and Shaw, 1995). This concept has been criticized for its stringent nature, as it, for example conflicts with the observed incomplete lineage sorting and admixture between the genomes of some well-recognised species (for example lineage sorting in Ursine bears, Kutschera *et al*., 2014; and apes, Mailund *et al*., 2014; and introgression between chimpanzees and bonobos deManuel *et al*., 2016; within gulls; Sonsthagen *et al*. 2016; and geese, Ottenburghs *et al*. 2017).

In contrast, other evolutionary biologists set the threshold for recognition of new species, much lower (*i.e.* so-called 'splitters', the past antonym of 'lumpers') whose approach is usually via the use of the Phylogenetic Species Concept (PSC). The PSC defines species as *"the smallest aggregation of (sexual) populations or (asexual) lineages diagnosable by a unique combination of character states"* (Wheeler and Platnick, 2000). This method of classification is much less stringent and it could be argued that any intra-specific population genetic structure should result in the fulfilment of the requirement of "*a unique combination of character states*". It has therefore been criticized for increasing the number of recognized species beyond what would seem justified, known as 'taxonomic inflation' (Heller *et al*., 2013; Zachos 2013; Zachos *et al*. 2013).

Recently, Gippoliti *et al.*, (2017) describe the opposing argument that 'taxonomic inertia' is actually more detrimental to conservation, highlighting the case of African ungulates. They argue that the history of African ungulate taxonomic classification has been dominated by 'lumpers' who, when faced with difficult taxonomic decisions, have avoided the situation by assigning a large number of subspecies or genetic lineage labels. According to the authors, this has led to a disproportionately small number of ungulate species being recognised in Africa (despite Africa being by far the leading continent in terms of recognized ungulate species richness (Heywood 2010)). Key to the argument of Gippoliti et al. (2017) is a survey by Morrison *et al.*, (2009), which showed that taxonomic splitting has a positive effect on conservation. Morrison *et al.*, (2009) identify numerous situations where a change in taxonomy has led to increased conservation efforts. One representative example is the California gnatcatcher, *Polioptila californica*. Morrison *et al.,* (2009) highlight the increase in conservation funding (better habitat protection and monitoring programs) that this species received after recognition of its species status. However, a change in protection (conservation) in this study was defined in Morrison *et al.,* (2009) as "*increased or decreased*

monitoring of any kind", as well as "*increased or decreased funding for research on the respective organism"*. This argument only considers the organism in question, not conservation actions as a whole. This overlooks an obvious concern, namely that conservation resources are finite (although not necessarily constant), and that resources allocated to one species do not necessarily benefit others. This is the 'Agony of choice' argument (Isaac, Mallet and Mace, 2004), which refers to the greater challenge of assigning limited conservation resources between higher numbers of taxa. Gippoliti *et al.*, (2017) also state that there is "no evidence for negative effects of taxonomic splitting on conservation". It could be argued, however, that this hypothesis would be very difficult to empirically support either way. It is not currently known precisely how much is being spent on conservation globally (McCarthy et al., 2012), let alone the relative amounts that are being spent on each taxonomic group. In an ideal scenario, all units of diversity would be conserved however, even in countries that allocate a relatively large budget to conservation efforts, this is rarely possible (Malaney and Cook, 2013), and even a prioritization approach may not be being practised (especially when it conflicts with other political priorities [Migration, Denver Post]). It therefore seems very likely that conserving the eleven species of klipspringer proposed by Groves and Grubb (2011) as separate taxa would require more resources than conserving the one klipspringer species, *Oreotragus oreotragus*, commonly recognised (e.g. Kingdon, 2013). In conservation (specifically in the IUCN context), a particular machinery comes into play when a new species becomes known, including making species status assessments, a species survival plan including *in situ* and *ex situ* measures (if deemed necessary for the species). All of these obviously require resources, and this is before even expending resources on the actual, practical conservation measures for the species.

Another argument for why over-splitting may be detrimental for particular taxonomic groups, Frankham *et al.*, (2012) focused on three widely used species concepts: the Biological (Mayr 1942, 1963), the Evolutionary (ESC; Simpson, 1951, 1961; Wiley, 1978) and Phylogenetic (Eldredge and Cracraft, 1980; Cracraft, 1997) Species Concepts. Frankham *et al.*, (2012) emphasised the point that diagnosably different population units are not intrinsically reproductively isolated (the importance of reproductive isolation is discussed in detail later), and that this is particularly relevant for small, allopatric populations. This is because the time in which a population attains alleles that make it distinguishable in e.g. multivariate genetic space will be proportional to their effective population sizes (Ne), and may be very short if Ne is low. The implication of this is that populations of the greatest conservation concern may be more likely to be diagnosed by the PSC. It should be noted that this argument is only referring to the conservation implications of the species concept used, and not an assessment of which is "correct".

It seems clear that both "taxonomic inflation" and "taxonomic inertia" could be detrimental to conservation. This is because implicit in those descriptors is an assumption that the populations in question have been artificially "grouped" more or less than what would be ideal under any given criterion (also keeping in mind that different criteria might not lead to the same "ideal" grouping!). Conservation (and in particular its genetic component) is predominantly a pragmatic discipline,

which for populations threatened with extinction, a primary concern is assessing whether re-joining populations (and therefore gene flow) is possible and deciding whether those populations *should* still be connected. These decisions are usually based on taxonomy, with the assumption that it is related to whether the populations are likely to be reproductively isolated, and to what extent they have unique adaptations to their local environment. However, this assumption may be correct or incorrect, depending on the premise of the species criterion used (see "*WHICH SPECIES CONCEPT BEST CONSERVES ADAPTIVE POTENTIAL?"*). This raises three important distinctions that need to be made when a decision is reached about what constitutes a species:

1. Distinguishing species
2. How they are diagnosed
3. Classification, i.e. how they are ranked

Point one is an ontological question, i.e. what one considers a species to actually *be*. Point two is a question of implementation: a technical/financial hurdle that is imposed based on the species concept that is chosen. Point three could be referred to as a "convention of organization", and depends on where any given organization chooses to delimit taxonomic boundaries. This framework links to the difference between a concept and a criterion, two terms that are frequently conflated in species discussions: A species concept relates to point one, and a species criterion relates to point two (De Queiroz 1998).

Some authors may argue that certain criteria are invalid because they do not identify units that they believe to be "real" species, however this can be countered by defining criteria as a concept, and thereby essentially redefining what a species *is* to fit in with a given criterion. It has been argued that all species concepts have a single common concept, namely that species can be equated with "segments of population-level lineages" (De Queiroz 1998; De Queiroz 1999), or groups of organisms with their own "independent evolutionary fate and historical tendencies" (Mayden, 1997). Under this framework, the so-called General Lineage Concept (GLC), it is argued that alternative species concepts are either variations of the GLC, or criteria of it. While this is a compelling argument, in the sense that it relates to ontology, it could still be considered a matter of opinion.

In an attempt to make the definition of species less arbitrary, increasingly sophisticated methods have been produced to delineate species. Developments in coalescent theory has allowed for the investigation of lineage diversification (Yang, 2015). Other methods for molecular species identification include Bayes factor-based species delimitation (Grummer et al. 2014). These methods, based on different criteria/theories, test species boundaries in a comparative way (Toussaint et al. 2016). However, the multispecies coalescent has also been criticised for only being a method to "delimit structure, not species" (Sukumaran and Knowles 2017).

Further discussion on which of the various species concepts is "correct" remains outside the scope of this manuscript. Rather we seek to ask if, and how, genomic data have influenced the operational nature of the various species concepts. Specifically, has the increasing resolving power of genomic tools i) been used to invoke the chosen species concept (we focus on the PSC and BSC) more readily,

or, ii) led to a more conservative approach to species delineation due to the complex interaction of admixture, incomplete lineage sorting, and demographic history that is increasingly being detected. We also revisit the question of the link between species concepts and adaptive potential, and whether new genomic data has had an influence on this question. We focus on case studies from the recent literature (Table 1), which highlight how species delineations have been applied to date. These studies either use what could broadly be described as the BSC or the PSC (here synonymous with "lumping" and "splitting", respectively) in order to justify their species delineations.

Table 1. Summary of the genomic evidence used in our case studies

Study	Reference	Genomic resources	Analyses	Species criteria used
Newly proposed species				
Giraffe	Fennessy et al (2016)	One draft genome	• Phylogeny • Genetic structure (Using 7 intron loci and mitochondrial DNA)	Unique genetic character states (PSC)
Orang-utan	Nater et al. (2017)	37 resequenced genomes	• Phylogeny • Genetic structure • Demographic history • Morphology (Genetic data from two, and morphological data from one *Pongo tapanulienis* individual[s])	Unique genetic and morphological character states (PSC)
Finless porpoise	Zhou et al (2018)	48 resequenced genomes	• Phylogeny • Genetic structure • Demographic history • Signatures of selection	Reproductive isolation (BSC)
Darwin's finch spp.	Lamichhaney et al (2017)	47 resequenced genomes, genomic data from 180 individuals from previous study	• Phylogeny • Morphology • Pedigree assessments • Demographic history • Phenotype-genotype associations •	Reproductive isolation (BSC)
Currently recognised species				
Stickleback spp.	Ravinet et al (2018)	27 resequenced genomes	• Phylogeny • Demographic history • Detection of introgression • Genetic structure • Signatures of selection	Species claim not made in this study, but well-recognised as different species with reproductive isolation, and ecological and phenotypic differences (BSC)

NEWLY PROPOSED SPECIES

Giraffe

Recently, Fennessey *et al.,* (2016), produced a draft genome for the giraffe (*Giraffa camelopardelis*), and analysed nuclear and mitochondrial sequences from 105 individuals from all currently recognized subspecies. In identifying four distinct genetic clusters they concluded that *"population genetic, phylogenetic, and network analyses of nuclear sequences demonstrate that the giraffe is genetically well structured into four distinct species"*. However, this conclusion was based on only two mitochondrial and seven intron loci. It contrasts with a previous genetic study of giraffe, which used 14 microsatellite loci from 381 individuals to identify

six distinct clusters (Brown *et al.,* 2007), without designating these clusters to species. Therefore, both studies were based on a relatively small number of loci that showed varying genetic structure but reached different conclusions. This could be explained by variation among loci with different realisations of stochastic lineage sorting, an effect that while still possible for large numbers of loci, is more likely to be observed in studies using relatively few (Orozco-terWengel *et al.,* 2011). The operational approach used in Fennessey *et al.*, (2016) could be described as conforming to the PSC, as the genetic structure was used to justify a "*unique combination of character states*" (i.e. nuclear alleles), present in each of the populations (or species).

Of all the examples presented below, the findings presented in Fennessey *et al.*, (2016) have probably received the most attention to date, reviving the debate on giraffe taxonomy and conservation. Bercovitch *et al.*, (2017) listed seven points of concern about the original authors' interpretation of their results. Their concerns included a criticism of the lack of concordance between mitochondrial and nuclear phylogenies, few loci, and a disagreement that assignment to separate genetic clusters was a sufficient indicator of species designation. They concluded that the study of Fennessey *et al.*, (2016) should only be regarded as one perspective on giraffe taxonomy. On the lack of power of the nuclear dataset used, Fennessey *et al.*, (2017) argued that *"Compared to microsatellite data, DNA sequences allow estimating divergence times"*. Fennessey *et al.*, (2016), however, did not estimate population divergence times, only sequence divergence times, which, incidentally, can also be estimated with microsatellites (e.g. Hey, 2010). The response by Bercovitch *et al.*, (2017) also highlighted different criteria for species delimitation than Fennessey *et al.*, (2016, 2017). Whilst Fennessey *et al.* (2016, 2017) advocate diagnosability using (neutral) genetic markers as the primary criteria for species delineations, Bercovitch et al. (2017) placed a greater emphasis on phenotypic and behavioural characteristics. They stated that: *"Coat color patterns are linked to specific gene complexes with mutations leading to variation subject to natural selection… Phenotypic traits regulate mating patterns and sexual selection that establish a foundation for the recognition species concept'"*.

Ultimately, Fennessey *et al.,* (2016) used limited genetic data to detect genetic structure and sequence divergence criteria, which were then equated with species divergence by applying the PSC. However, the process of lineage sorting under plausible demographic and selection models was not considered, nor their influence in the context of the limited number of markers used. A follow-up study using a larger set of nuclear markers has since been carried out, which confirms that gene-flow between the four proposed species is very low (Winter et al. 2018). However, it appears that in this situation the argument is predominantly of an ontological nature, and so may not have run its course yet.

Orang-utan

Nater *et al.*, (2017) recently described the genomic diversity of a population of orangutans from the species' southernmost range limit in Sumatra (Batang Toru). They concluded that the Batang Toru population was sufficiently distinct to warrant being named a new species. This conclusion was based on morphometric, behavioural and genomic evidence from 33-37 individuals (the morphological

analysis could only use a single Batang Toru specimen). Using Approximate Bayesian Computation modelling of demography, it was estimated that the northern Sumatra population split from the older Batang Toru ~3.4 million years ago (mya), but maintained gene flow until 10-20 thousand year ago (kya). The authors also point out that there are many instances of ongoing gene flow between taxa that are recognised as distinct, well-established species. In light of this, Nater *et al.*, (2017) use the species definition that describes species as "*a population (or group of populations) with fixed heritable differences from other such populations (or groups of populations)",* effectively invoking the PSC.

The morphological evidence which led to the conclusion of a new orang-utan species was based on a single specimen from the population in question (and genomic evidence based on two). Any criticisms of the validity/robustness of this conclusion could be centred around the question of whether a single specimen can be considered representative of the whole population. Nater *et al.*, (2017) point out that numerous species have been identified based on a single type specimen in the past. Based on genomics, the authors were able to show that these two orangutan populations had fixed heritable differences with an estimated termination of gene-flow from/to the proposed new species 10 – 20 kya. Yet, Nater *et al.* (2017) did not assess if these SNPs were associated with adaptive differences between the populations. Thus, although Nater et al. (2017) used genomics to enhance their power to apply the PSC with greater resolution, they did not use it to attempt to understand the speciation process in any mechanistic sense. The conclusions reached by Nater *et al.*, (2017) has not been accepted by all in the scientific community, particularly by proponents of the BSC (e.g https://whyevolutionistrue.wordpress.com/2017/11/03/a-new-species-of-orangutan-i-doubt-it/). Nater *et al.*, (2017) pointed out that determining if these populations are reproductively isolated or not is not possible, due to their allopatric distribution. One potential solution that was not used by Nater et al. (2017) is the Tobias criteria (Tobias et al. 2010). This uses sympatric species pairs to set thresholds for delineating allopatric taxa. It seems likely that despite the large number of features investigated, and analytical methods applied, this approach will still fall short of the expectations of many proponents of the BSC.

In short, the orang-utan paper represents a case in which a large panel of the genomic tools available have been applied to address the question of population divergence. While presumably adding detailed information about the historical processes, it does not attempt to analyse adaptive differences, nor to answer whether maintaining these two populations of orang-utan as separate would maximize the adaptive potential going forward.

Finless porpoise

Zhou *et al.*, (2018) investigated speciation in finless porpoises, which have traditionally been classified as a single species, *Neophocaena phocaenoides*. Finless porpoises exist as three geographic populations or subspecies, two marine (Indo-Pacific) and one freshwater population (Yangtze River). Zhou *et al.*, (2018) identified several candidate genes related to hypoxia that show strong evidence of directional selection. They also estimated divergence of the Yangtze River population at 5,000 – 40,000 years ago. These findings led them to conclude that

"*significant population differentiation, lack of gene flow, and unique adaptive divergence in the Yangtze finless porpoise make it clear that the Yangtze finless porpoise is genetically and reproductively isolated from its marine counterpart and thus represents an incipient species*".

The main aspect that differentiates the porpoise case study from that of the orangutan is the term "*unique adaptive divergence*". By identifying selection signatures in several candidate genes that are the result of diversifying selection to two different ecosystems, Zhou *et al.*, (2018) found plausible mechanistic evidence for an instance of incipient speciation. Whilst the orang-utan study by Nater *et al.*, (2017) showed phenotypic differences between the two proposed species, no evidence was presented to demonstrate that this divergence was adaptive, and therefore driving speciation. This highlights the issue that, although genomic methods for identifying selection in natural populations has advanced considerably over recent years, it is still challenging to do this with limited numbers of samples.

Darwin's finches

Lamichhaney *et al.*, (2017) documented a remarkable example of hybrid speciation from its origin to reproductive isolation in a hybrid between two Darwin's finch species (*Geospiza fortis* and *G. conirostris*). This hybrid lineage was shown to breed endogamously from the second generation onwards, with transgressive segregation of bill morphology, a trait that is known to be under strong selective pressure in these species. This study demonstrates that reproductive isolation can occur rapidly, in as little as three generations. This species classification was therefore based on reproductive isolation of the new hybrid finch lineage from its parent lineages, aka the BSC.

Prima facie, the question of a new species of Darwin's finch seems very simple: These species exist in sympatry, and were observed to stop interbreeding, a situation clearly fulfilling the criteria of distinct species under the BSC. However, Hill and Zink (2018) firstly notes that three to four generations may not be enough time to determine if the new lineage is ephemeral or not, and secondly that phenotypic differences observed may be highly plastic. The conclusions of Lamichhaney *et al.*, (2017) are strengthened by the fact that they also investigated the genetic basis for bill dimension, a morphological trait that is implicated in driving ecological success and reproductive isolation of the big bird lineage. By observing correlations between the ALX1 and HMGA2 loci with morphometrics, they were able to use genomics to provide evidence for genetic adaptation to a new environment. It seems unlikely that the level of observational evidence that they used will be practical for most wild species, a common criticism of the practicality of the BSC (Amato and Russello, 2014). However, there are genomic approaches that can bypass these challenges for many taxa. For example, relatives, pedigrees, and local ancestry tracts can be identified so that reproductive isolation over the last few generations can be inferred from genetic data (e.g. as carried out in humans, Ko and Nielsen, 2017). This could serve as an alternative to observational studies.

This is not to say that there are not conceptual criticisms that can be made of the BSC regardless of how it is operationalized (e.g. related to instances of viable hybrids between organisms well-recognised to be different species (Nater *et al.,* 2017)). As discussed earlier, a full discussion of this is beyond the scope of this manuscript, however, genomic tools are at least allowing us to be able to better quantify and understand the relevance of these instances (even when we only have low coverage data or few individuals, Schaefer et al., 2017).

Genomic and other data increasingly show that these hybridization and introgression events can no longer be classed as a rare or insignificant: they are now being recognised as both common and important evolutionary mechanisms, including sometimes being implicated in the adaptive advantages to a newly colonised environment (e.g. invertebrates, Pogson, 2016; plants, Ru *et al.,* 2016; and vertebrates, Barbato *et al.,* 2017).

THE ROLE OF HYBRIDISATION IN SPECIES DESIGNATION

Hybridization is ubiquitous in nature. Sixteen percent of bird species (Ottenburghs et al. 2015), 6% of European mammals and at least 25% of vascular plants (Mallet 2005) are thought to hybridise. Ravinet *et al.*, (2018) investigated signatures of divergence and introgression in a species pair: The Pacific Ocean three-spined stickleback (*Gasterosteus aculeatus*) and the Japan Sea stickleback (*G. nipponicus*). These are well-recognised as different species that have sympatric distributions and crosses showing male hybrid sterility (Kitano, Mori and Peichel, 2007). However, despite the high differentiation, relatively large divergence time (0.68 – 1 mya) and hybrid sterility, ongoing gene-flow and localised introgression could nonetheless be detected (maintained in small regions within the genome). Although the authors are not making a new species claim, this observation of introgression despite the considerable divergence time is highly relevant to the speciation question.

This situation provides challenges for both the PSC and the BSC. How infrequent do hybridization events have to occur before we consider two biological entities to be different species? Does it make a difference if such hybridization is sex-biased? How does regional variation in hybridization rates influence this inference? The BSC currently has no answer to these questions. Likewise, for the PSC, "fixed heritable differences" will be immediately mixed in hybrid individuals. Therefore, temporal or spatial variation in hybridization could lead to transient or spatially varying species classifications.

Due to the increasing recognition of the pervasiveness of hybridization and introgression among recognised species, they are becoming important phenomena to consider when making taxonomic decisions. The idea that hybridization may play an important role in evolution was initially explored by botanists and appears to be particularly important for plants, with approximately 10% of plant species thought to hybridize (Yakimowski and Rieseberg, 2014). Hybridization is also particularly common in invasive species (Ellstrand and Schierenbeck, 2000), likely due to hybridization allowing adaptive introgression of beneficial traits between the taxa (Martin, Bouck and Arnold, 2005, 2006).

However, widespread hybridization is not limited to plants and has played an important role in the adaptive radiation of *e.g.* Heliconius butterflies (Dasmahapatra *et al.,* 2012). These butterflies are of particular interest in speciation research because of their huge diversity, with varying rates of hybridization (Van Belleghem *et al.,* 2017). Their genomes contain what has become known as "genomic islands of divergence" (Nadeau *et al.,* 2012). Originally identified in *Anopheles* mosquitos (Turner, Hahn and Nuzhdin, 2005), the origin and role of these islands was originally interpreted as regions of selection and reduced introgression between divergent populations, although it is increasingly being realised that there are processes other than population divergence that may lead to these patterns (Cruickshank and Hahn, 2014; Wolf and Ellegren, 2017).

Hybridization complicates taxonomy when we consider that speciation rates, and levels of subsequent hybridization vary considerably between taxa. The proposed new species of Darwin's finch described above showed transgressive segregation in bill morphology and was ecologically successful. This ongoing finch radiation is predominantly based on a behavioural trait (i.e. mate choice). Finches imprint on features of their parents early in life, and choose mates based on bill size and shape, and body size and song. The driving force behind the speciation events here is therefore a complex mating behaviour. While these adaptive traits (at least in the case of bill dimensions) are correlated with detectable genetic variation, it is their effect on the behaviour phenotype that is relevant for reproductive isolation and species designation in these taxa. It seems fair to assume that if the observational data were available, this situation would be representative for most taxa with complex mating behaviour. However, this is in stark contrast to many other taxonomic groups, which can take far longer to develop reproductive isolation. For example, hybridization in marine invertebrates may be extreme. One study found hybridization between two cryptic species of sea squirt (*Ciona intestinalis*) with an average synonymous sequence divergence of 14.4% (Roux *et al.,* 2013). Rates of introgression in *Ciona* were relatively low, variable among loci, and unidirectional, consistent with a situation of multiple genetic incompatibilities throughout the genome, suggesting that genetic incompatibility was developing, albeit very slowly. It would be interesting to use genomics to investigate signatures of selection in these *Ciona* populations, to see the extent to which adaptation can be detected, and how it reflects the taxonomy.

Previously, we might have written off these examples of extreme hybridization as being exceptional, however this explanation is becoming more difficult to abide. As we can see from the stickleback example above (Ravinet *et al.,* 2018), the phenomenon is not limited to invertebrates. In fact, whole genome data are detecting instances of introgression in many species and in unprecedented detail. For example, most non-African humans have 1-2% Neanderthal ancestry (Green *et al.,* 2010; Prüfer *et al.,* 2014), and a number of human populations have Denisovan ancestry that is thought to have adaptive significance for adaptation to extreme altitude (Reich *et al.,* 2010; Meyer *et al.,* 2012; Prüfer *et al.,* 2014). Such patterns of introgression are mirrored in non-human primates, with evidence of multiple occurrences between bonobos and chimpanzees during the past 550,000 years (De Manuel *et al.,* 2016).

These observations complicate the matter of species delineation, because they suggest that complete reproductive isolation can be withheld for extremely long periods of time in some taxa (in the case of *Ciona*, for greater than three million years of divergence in isolation). It could be argued that this is just the BSC impartially reflecting the variable speciation rates that occur in nature, however some taxonomists (e.g. with well-known mammalian groups) clearly find such observations problematic as these instances do not tend to be reflected taxonomically (e.g. between brown and polar bears, coyotes and wolves). Some concepts may regard hybridisation as a "consequence", while others think of it as a defining characteristic. However, hybridisation does not only complicate species designation for the latter. Hybrids may not initially seem relevant to the PSC, but hybrid zones between two different taxa diagnosed using the PSC would create a gradient of alleles, such that the sampling scheme (across the geographic space as well as the genome) and population comparison chosen would dictate whether taxa would be diagnosed as different. This present a challenge, not only for diagnosing different units, but also for describing what those things are from an ontological point of view.

ARE THE SPECIES CONCEPTS OPERATIONAL IN THE GENOMIC ERA?

There are therefore challenges in operationalizing species concepts, but is this more the case for some rather than others? And how has genomic data facilitated operationalisation for each concept? The PSC is easier to test in most cases, and Groves (2013) argued that *"the PSC offers the only criterion for species recognition that is testable, as a scientific proposition should be."* However, it might be questioned in what sense the PSC is testable. And if so, is it the only species delineation approach that is?

As argued by Groves (2013), the PSC is "testable", however when we do this we must be cautious that we are not engaging in an oversimplification. The application of a testable threshold does not represent progress if that threshold does not reflect the label that we are trying to establish. Genetic differentiation among populations can be greatly influenced by demography, including changes in population size. Genetic structure has been observed to considerably decrease among brown bear populations (*Ursus arctos*) in just 1.5 generations (Hagen *et al.,* 2015), and genetic structure substantially increased over only eleven years (approx. five generations) in Coachella Valley fringe-toed lizards (*Uma inornata*; Vandergast *et al.,* 2016). These examples are not intended to demonstrate that speciation does not occur over short time periods, but simply that genetic divergence and population structure may be highly transient, which many people would argue should not be the case for speciation. Genomics allows for a huge increase in the power to detect population structure because of the much larger number of loci available. This has the effect of enabling the identification of very fine-scale population genetic structure, and consequently more 'fixed heritable differences' between populations. 'Splitters' would presumably interpret this added genomic information as an increase in power of detecting incipient speciation, whereas 'lumpers' would presumably interpret these as 'type 1 error' species. In this regard then whether genomics has revolutionised our ability to identify new species depends on the species concept being applied. Genomics has

also allowed for a huge increase in the power to describe demographic histories (e.g. Nater *et al.,* 2017), and this information is important to present alongside that of genetic structure when making a species claim, so that that claim can be assessed in its full context.

All but one of the examples discussed here have used genetic structure as a part of their evidence, however it is notable that the porpoise (Zhou *et al.,* 2018), orangutan (Nater *et al.,* 2017) and stickleback (Ravinet *et al.,* 2018) studies also include demographic analysis, whereas the giraffe study (Fennessy *et al.,* 2016) did not. Genetic structure does not distinguish between isolation and migration and so is very difficult to interpret on its own. In addition, the first three studies above used a methodology and dataset that enabled them to estimate genetic structure that was representative of the whole genome. As we can see from the stickleback example (Ravinet *et al.,* 2018), and the discussion on introgression above, using genetic structure based on a small number of loci can be misleading: Even species with high genomic divergence may have introgressed regions that will give a very different perspective of the taxonomy - and even without gene flow incomplete lineage sorting can generate a high proportion of "wrong" gene trees (Jarvis et al. 2014).

An understanding of population structure can be important for conservation, but it is important to understand its limitations. Frankham *et al.*, (2012) argued that species delineations need to be relevant to the point at which populations have/have not become reproductively isolated (which is not necessarily related to genetic structure), in order for them to minimise the risk of inbreeding and outbreeding depression and maximise the benefits of gene-flow. These arguments led the authors to recommend that only substantial reproductive isolation be used to define species (for outbreeding sexual organisms) in conservation. Amato & Russello (2014) commented on this paper, with their main critique being the difficulty of operationalising the BSC. Frankham *et al.*, (2014) countered that reproductive isolation generally arises from adaptation to different environments and/or outbreeding depression caused by fixed chromosomal differences, both of which can be detected (albeit requiring a more technically challenging approach than a structure analysis). They stated that "*Divergence should be protected when it reflects adaptive differences, but countered when it threatens populations.*" The authors were therefore arguing that the BSC is be a better proxy for adaptive potential than the PSC. It is important to note that this argument is predominantly based on the BSC being a better tool for recognising conservation units, and therefore is not addressing its ontological relevance. Nonetheless, adaptive potential *is* important if we want to conserve populations that are able to adapt to changes in their environment. However, is it true that the BSC preserves adaptive potential better, and if so, are there limits and/or exceptions to this?

WHICH SPECIES CONCEPT BEST CONSERVES ADAPTIVE POTENTIAL?

Adaptation to novel ecological opportunities is one of the main drivers of speciation (Van Belleghem *et al.,* 2017), and predicting the capacity of taxonomic groupings to respond to changing environments is therefore crucial to their conservation (Eizaguirre and Baltazar-Soares, 2014). The Darwin's finch example

above is a clear demonstration of the potential of hybridization to produce a population with unique adaptive potential. However, this hybridization and introgression may have a confounding influence on species delineations (particularly for the BSC), which is exacerbated when we also consider the adaptive advantage that introgressed genes may bring. This process, adaptive introgression, poses a challenge to the claim that the BSC is a good proxy for adaptive potential. Even very low levels of introgression can have a large effect on the adaptive potential of the recipient population; adaptive genetic variation has the potential to move to high frequencies very quickly in a population (Maynard Smith and Haigh, 2008). In addition, the adaptive potential of the introgressed material may vary between the donor and recipient populations, depending on factors such as population size and selection regime. Therefore, in some situations, taxa designated by the BSC (even when allowing for very low levels of introgression) may be reflective of adaptive differences between them (e.g. the adaptive differences in the Darwin's finch example). However, in many situations it will not. For example, it seems highly likely that the two distinct populations of sea squirts (*Ciona*) (Roux *et al.*, 2013) have accumulated considerable adaptive differentiation in their three million years of divergence in isolation, regardless of the fact that gene-flow has now been re-established. This gene-flow would preclude these as separate species under the BSC, and therefore (unlike with the finches) the taxonomy would not reflect the adaptive differences between populations/species. Hence, the BSC will better represent adaptive differentiation in some comparisons than in others, and this may be biased towards taxonomic groups with particular life-history traits. It should also be noted that this is no less the case for the PSC. If our goal is to conserve adaptive potential in an unbiased way across all taxa then this is a crucial point to consider. Many scientists argue that maximizing phylogenetic diversity will indirectly capture functional diversity (Vane-Wright *et al.,* 1991; Faith 1992; Winter et al., 2013). However, a recent study by Mazel *et al.*, 2018 has shown that phylogenetic diversity does not reliably capture functional diversity.

This raises the question of why not simply measure adaptive potential directly? Genomics is starting to allow us to do this. For example, Zhou *et al.*, (2018) identified evidence of selective sweeps in a number of genomic regions across the porpoise genome using a method that looks for distinctive patterns of allele frequencies along a chromosome (Nielsen *et al.,* 2005). Other commonly used methods for detecting selection include: 1. Identification of extended haplotypes that are at, or near fixation in a subset of individuals (Sabeti *et al.,* 2007), 2. Outlier methods that compare a model based on including versus excluding selection (Foll and Gaggiotti, 2008), 3. Attempts to identify correlations between SNPs and environmental variables (Coop *et al.,* 2010). In the porpoise example, Zhou *et al.*, (2018) found regions that have a plausible link to morphological characteristics that differentiate the two proposed incipient species. Applying these methods has the benefit of not requiring the assumption that adaptive differences are related to reproductive isolation or genetic structure, which, as described above, may be inaccurate. It should be noted however that tracking adaptive changes using genomics is challenging for many traits, especially those that have low heritability or are highly polygenic (Hoffmann, Sgrò and Kristensen, 2017). However, it is often hard to convincingly demonstrate selection on a given region of the genome

as in many cases it is only the regions undergoing strong haplotypic selection that will be detected in the analyses discussed above. Furthermore, demonstrating past selection may not necessarily be associated with contemporary or future adaptive potential of a genome/genomic region, given that selection pressures are dynamic. Finally, even if a genomic region can be identified as being under selection, determining the specific "cause" of this pressure can be highly challenging, particularly for non-model organisms.

We have argued that some species concepts may be more applicable (in terms of relating to adaptive potential) to some taxa than others. For example, reproductive isolation may be a useful criterion in the case of Darwin's finches, since it aligns with the behavioural, morphological and ecological differences between populations. For organisms like sea squirts, genetic distance and differentiation may be a better reflection of the differences that have accumulated over long periods of temporal and spatial isolation. The relationship between adaptive potential and species concept therefore seems to depend on the taxa being investigated. This does not necessarily mean that these are not good criteria, independently, for defining species. However, it certainly complicates conservation strategies that aim to maximise evolutionary potential, especially when only one is considered at a time. We would therefore caution against focusing on a single species concept, especially when the taxa in question are of conservation concern. In this situation it is important to be very clear about which concepts are being invoked, and how the evidence presented supports them. It is important to incorporate multiple lines of evidence into taxonomic decisions (which is increasingly being done; Schlick-Steiner et al. 2010) however, this evidence can now theoretically be provided by entirely by genomics: 1. Morphological evidence can be identified via differentiation in developmental and structural genes, 2. Biogeographic evidence can be provided using sophisticated genome-scale modelling, 3. Behavioural differences can be inferred by identifying genes associated with behaviour, mate-choice, and also by detecting sex-biased demography, 4. Ecological evidence is available in the form of genomic signatures of selection to environmental factors, 5. Reproductive compatibility can be observed as sex chromosome compatibility/incompatibility, chromosomal structure, and epigenomic transmission. In lieu of a definitive conclusion as to the most appropriate species concept to be used, best practice would be to investigate as many of the above lines of evidence as possible, and to apportion ones confidence in a species designation based on the combined weight of all of them. Recently, Kitchener et al (2017) introduced the concept of a 'traffic light' system for evaluating the strength of evidence of the above 5 categories of species differentiation, which may provide a pragmatic approach to evaluating genomic data in specific definition if applied sensibly.

One thing that both 'splitters' and 'lumpers' seem to agree on is that it is preferable that conservation decisions are based on sound scientific evidence. Any 'planning blight' due to taxonomic uncertainty can be detrimental to conservation, and renders decisive action more difficult. However, while we still have some way to go before genomic techniques reach their full potential as a diagnostic tool for species delineation, if the ultimate goal of conservation is to preserve adaptive potential, genomics is now allowing us to gain a better understanding of this in

wild populations. A pragmatic approach could be to use genomic tools to characterise adaptive potential regardless of the species concept, or even without invoking a species concept at all. However, answering the question of whether and to what extent such studies *should* focus on adaptive potential is a separate challenge.

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