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COMMENT

When is a hybrid a hybrid? A counter-reply to Wallace *et al.*

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In a reply to a brief citation of their paper (Wallace *et al*. 2004), Wallace *et al*. (2006) re-analyse their data using the same methods as we did (Engel *et al*. 2005) to conclude again that an ecad of the brown alga *Fucus* sp., which occurs only in a particular type of habitat, was a hybrid. While we wholly agree that the ecad individuals (called 'muscoides-like') may be *putative* hybrids, their conclusions were — and remain — confounded with other, alternative explanations. Here, in response to their Reply (Wallace *et al*. 2006), we wish to clarify our reservations more fully and offer some reflections on identifying hybrids in species complexes.

Identifying hybrids in species complexes requires genetic markers that are diagnostic for the species. In the absence of diagnostic alleles, as might happen in complexes of young taxa undergoing speciation (e.g. *Fucus* spp., Serrão *et al*. 1999), intermediate allele frequencies can provide evidence for hybridization if the genetic variability of the parental species is well characterized. However, an appropriate sampling scheme is essential for ruling out the more parsimonious alternative explanation that intermediate genotypes are a subset of the total variability of one of the parental species. In addition, sympatry of hybrids and parental species is required to distinguish hybridization from the much simpler process of genetic differentiation of populations separated geographically or by habitat. However, the sampling scheme that Wallace *et al*. (2004) used did not meet these requirements. First, the variability, characterized at four microsatellite loci, appears underestimated in one of the 'parental' taxa, *Fucus vesiculosus*, due to a sample size ($n = 33$) that was less than a third that of the other 'parental' taxon, *Fucus spiralis* ($n = 113$). Yet, the ratio of intra- vs. intertaxon genetic variability was high in their study, suggesting that clear genetic entities may be difficult to define and that estimates of allele and genotype frequencies may be biased. However, the hybrid status of the muscoides-like taxon was entirely based on indices (e.g. 'allele frequencies, observed heterozygosities, positive [*sic*] F_{IS} estimates and genotype clustering' Wallace *et al*. 2006), although these estimates lack robustness. Second, the putative hybrids came from sites where both putative parents are not always present, therefore hindering robust characterization of the genetic variability and signature of the parental species: extensive sampling throughout the possible ecological and geographical range is essential under nonsympatric conditions where the location(s) of the source (i.e. parental) population(s) is(are) not known. A new analysis using the same samples, as Wallace *et al*. (2006) propose in their Reply, will never correct for problems arising from an inadequate sampling scheme, and cannot validate their previous conclusions.

Concerning their new analysis, the two parental and the hybrid groups were defined a priori based on morphological criteria. However, the genetic signature of these groups was determined a posteriori. This means that the groups do not necessarily form cohesive genetic entities. Indeed, the new analysis using STRUCTURE (Pritchard *et al*. 2000), as pointed out by Wallace *et al*. (2006), shows that both 'parental species' comprise many genetically intermediate individuals and that some muscoides-like individuals could be classified as *F. spiralis* or *F. vesiculosus*. Disregarding the problem of the circular reasoning employed in detecting genetic entities and potential hybridization, the presence of genetically intermediate individuals in one or both parental species has — as we discuss in our original paper (Engel *et al*. 2005) — two explanations: (i) ancestral polymorphism, and (ii) interspecific gene flow (i.e. introgression). Ancestral polymorphism is certainly plausible, particularly in the case of a complex of species that have diverged only recently or are continuing to diverge (see Serrão *et al*. 1999). Introgression is also plausible if reproductive barriers are weak and when hybrids are fertile (as in Engel *et al*. 2005). However, the muscoides-like individuals (i.e. the putative hybrids), were sterile.

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