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## REVIEW ARTICLE

### The “Evolutionary Significant Unit” concept and its applicability in biological conservation

L. P. CASACCI, F. BARBERO\*, & E. BALLETO

*Department of Life Sciences and Systems Biology, University of Turin, Italy*

#### Abstract

Although most conservationists claim to protect “species”, the conservation unit actually and practically managed is the individual population. As resources are not unlimited, we need to focus on a restricted number of populations. But, how can we select them? The Evolutionarily Significant Unit (ESU), first conceptualised by Ryder in 1986, may offer some answer. Several definitions have been proposed for the ESU, but all make reference to units “whose divergence can be measured or evaluated by putting differential emphasis on the role of evolutionary forces at varied temporal scales”. Thus, an ESU might be fully identical with a “species”, or a “species” could be composed of multiple ESUs. On the other hand, an ESU might comprise single/multiple populations exchanging a degree of gene flow, such as meta-populations. In an attempt to show strengths and weaknesses of ESU concepts, we present here, among several others, some case studies on the myrmecophilous butterflies of the genus *Maculinea*. In particular, we analyse the apparently everlasting debate about *Maculinea alcon* and *M. rebeli*, whose separation into separate species has been accepted by many authors, on mainly ecological criteria, but has not been fully supported by molecular analyses. We also discuss how the tight association with host ants may have driven selection for increasingly more strictly adapted *Maculinea* populations, arguably deserving specific taxonomic identity. Finally we discuss how current DNA analyses may fail to detect critical

information on differences between taxa recently originated by the action of separate adaptive processes, which non-molecular studies can sometimes reveal. We conclude by discussing some current and often conflicting taxonomic trends, in their relationships with conservation policies.

**Keywords:** *Evolutionary Significant Unit, butterflies, Maculinea, conservation, species concepts*

\*Correspondence: F. Barbero, Department of Life Sciences and Systems Biology, University of Turin, Turin 10123, Italy. Tel: +39 011 6704538. Fax: +39 0116704508. Email: francesca.barbero@unito.it

Luca Pietro Casacci and Francesca Barbero contributed equally to this manuscript.

Luca Pietro Casacci, Department of Life Sciences and Systems Biology, University of Turin, Turin 10123, Italy. Tel: +39 011 6704538. Fax: +39 0116704508. Email: luca.casacci@unito.it

Emilio Balletto, Department of Life Sciences and Systems Biology, University of Turin, Turin 10123, Italy. Tel: +39 011 6704515. Fax: +39 0116704508. Email: francesca.barbero@unito.it

***Running head: ESUs in biological conservation***

## **Introduction**

### **The conservationist's dilemma: What should we protect?**

It is widespread belief, in biological conservation, that what should be protected are species (Mace 2004). Even setting aside the many important theoretical issues and definition problems related to this concept (Hausdorf 2011; Simonetta *in this issue*), it remains that with a few though notable exceptions, the IUCN (2010), as well as, among the others, the European Union, with its “Birds”

(2009/147/EC, 30 Nov 2009) and “Habitats” (92/43/EEC and later amendments) Directives, (almost) exclusively recognise this taxonomic rank. A number of species are deemed threatened at European level, even though what is a “species”, for a taxonomist or a conservationist based in a given Country or geographical area, may be a “subspecies” for another based elsewhere. Apart from this and other theoretical issues related to varying taxonomic philosophies, this approach is not necessarily the best, or the most pragmatic.

### **ESU and other related concepts**

At global level, other Countries follow different approaches and several are trying to protect so called “Independent Conservation Units” (ICU), rather than species, at least as the latter are normally defined. In the USA, for instance, the “Endangered Species Act” (1973, see Waples 1991) makes reference to “species” including any number of “Distinct Population Segments” (DPS), the latter a concept having strongly pragmatic basis and often explicitly refusing any theoretical definition, at least in its application (Pennock & Dimmick 1997). The only prerequisite of a DPS is that it should be reproductively at least partially isolated by some physical barrier. Some authors or State Agencies make reference in this respect to ill-defined Ecologically Significant Units, such as the salmons living in a given lake, or “distinct” riverine system. Taken in this way, DPS can easily encompass all conservation needs, from protecting viable local populations of periodically harvested species (shooting, angling), to preventing local extinctions, on a State by State, or even County by County, basis.

Of course, in a man-dominated “Anthropocene” (Settele & Spangenberg 2013), the “unit” actually and practically managed can only be the individual population. Ideally, in case resources were unlimited, we might try and protect all populations of any given species, but this scenario is often not realistic and we need to focus on a restricted number of local populations. But, then, how to select them?

The Evolutionarily Significant Unit (ESU), conceptualised by Ryder (1986) as a conservation unit below the species level, but theoretically applicable to a wide range of taxa, may offer some answer. Indeed, the ESU notion was conceived to provide a theoretical background for prioritising taxa for conservation purposes and in the face of economic constraints, as well as of the inability of taxonomy to reflect apparent genetic diversity (Moritz 1994a,b). On this basis, an ESU might be fully identical with a “species” (i.e. a species encompasses one evolutionary lineage), or a “species” could be composed of multiple ESUs. On the other hand, an ESU lineage might comprise single/multiple populations, as well as groups exchanging a degree of gene flow such as meta-populations, this being always dependent on specific life histories (Fraser & Bernatchez 2001). Similarly to the “species” concept, several and sometimes contrasting definitions have been proposed for ESU in the course of time (Ryder 1986; Waples 1991; Dizon et al. 1992; Moritz 1994a,b; Vogler & Desalle 1994; Fraser & Bernatchez 2001; de Guia & Saitoh 2007). All of these definitions, however, aim at defining an identical “entity” i.e. “segments of species [viz. evolutionary lineage] whose divergence can be measured or evaluated by putting differential emphasis on the role of evolutionary forces at varied temporal scales” (Fraser & Bernatchez 2001). Some current definitions are summarized in the Table I.

[Insert Table 1 about here]

The ESU notion has gained scientific support and has been adapted or linked to various criteria and scenarios. Some authors, in fact, have suggested that obtaining a fixed and universal definition of ESU, valid across all species, may not be feasible (e.g. Fraser & Bernatchez 2001). Since all ESU definitions possess both strengths and bits of weakness, authors have argued that differing approaches may work more efficiently than others, depending on cases and circumstances. This implies that designating ESUs should be done flexibly, on a case-by-case basis (Fraser & Bernatchez 2001). Traditionally, judgements on how distinctive a population should be, before it becomes eligible for being recognised as an ESU, were based on ecological, as well as on variously measurable genetic information, thereby trying to take into account its effective evolutionary

distinctness (see Ryder's but also Crandall et al. 2000 definitions in Table I). Definitions by some other authors (Waples 1991; Dizon et al. 1992; Vogler & Desalle 1994; Bowen 1998) tend to overlap with subspecies concepts. Some recent works, taking into account the ever increasing availability of genetic data, have suggested, or sometimes even tended to force, the adoption of criteria exclusively based on molecular phylogenies, while largely ignoring all other otherwise measurable adaptive components (Avice 1994; Moritz 1994a,b). Researchers are often prompted to assess supposedly neutral genetic variation, more or less combined with as supposedly adaptive nuclear DNA variation. Here we have a theoretical issue, because although mainstream notions of speciation mechanisms include evolution of separate adaptations in allopatric or peripatric conditions (see Mayr 1963; Provine 2004 for reviews), the ESU concept, which implies demonstrable adaptation, does not necessarily overlap, or can be applied, to every separate segment in a phylogenetic tree, whose divergence may be a consequence of other genetic mechanisms, unless otherwise demonstrated. Strictly speaking, populations or taxa apparently characterised only by genetic divergence should better be considered Conservation Significant Units (see Yuan et al. 2011), rather than ESUs.

We will now analyse some case studies, trying to show some strengths and weaknesses of ESU concepts, which, since our scientific work has mainly dealt with Lepidoptera, will be drawn from this Insects' Order.

### **The ESU: Some insights from butterflies studies**

The field of insect conservation is littered with enormous challenges (Stewart et al. 2007). Among insects, butterflies possess well-known ecological preferences and respond to the action of drivers of change even more strongly and faster than other well-studied taxa, such as birds and vascular plants (Warren et al. 2001). Thus, butterflies represent a good indicator group for other insects taxa (Thomas 2005; van Swaay et al. 2010). More and more frequently, local extinctions have occurred even in nature reserves, where species are supposedly not facing any resources' shortage (New et al.

1995; Bonelli et al. 2011), so that many early attempts to conserve declining butterfly species have failed because of our inadequate understanding of their biology and causes of decline (Thomas et al. 2009). Among Lepidoptera, many taxa of conservation concern include entities which significantly diverge for molecular, morphological (i.e. wing patterns), ecological (i.e. differences in phenology etc.) and/or behavioural features.

### **When taxonomic variability does not reflect biological diversity in butterflies**

Current molecular studies have surely done a lot to improve our understanding of butterfly evolution, speciation, taxonomy and conservation priorities. A proportionally great number of ‘double’ (in one case triple) species have been identified by these methods and butterflies may be second only to Amphibians, or sometimes Mammals, in this respect. Cases such as those of *Zerynthia polyxena* ([Denis & Schiffermüller], 1775)/*Z. cassandra* Geyer, [1828]; *Pieris daphidice* (Linné, 1758)/*P. edusa* (Fabricius, 1777), *Leptidea sinapis* (Linné, 1758)/*L. juvernica* Williams, 1946/*L. reali* Reissinger, [1990]; *Melitaea phoebe* (Goeze, 1779)/*M. ornata* Christoph, 1893; *Melitaea athalia* (Rottemburg, 1775)/*M. nevadensis* Oberthür, 1904; *Coenonympha pamphilus* (Linné, 1758)/*C. lyllus* Esper, [1805]; *Polyommatus icarus* (Rottemburg, 1775)/*P. celinus* Austaut, 1879 (e.g. Porter et al. 1997; Dapporto 2010; Dincă et al. 2011a,b; Tóth & Varga 2011; Zinetti et al. 2013), to cite some of the most recently demonstrated, do not fit well into the ESU paradigm. These species, irrespectively of their generally strong genetic differentiation, apparently show too scarce ecological distinctness, within the same pair, to allow us to classify them as separate ESUs. It seems almost as though two or more species may form a single ESU, at least on the basis of the more adaptively restrictive definitions, as well as of our current understanding of their biology.

### **The case of *Maculinea* butterflies**

The five European Lycaenids of the genus *Maculinea* van Eecke, 1915 (*M. arion* (Linné, 1758), *M. teleius* (Bergsträsser, 1779), *M. nausithous* (Bergstrasser, 1779), *M. alcon* ([Denis &

Schiffermüller], 1775), *M. rebeli* (Hirschke, 1905)) are among the most well studied myrmecophilous butterflies and have become a model system for studies in the field of evolutionary ecology (Thomas & Settele 2004; Barbero et al. 2009b; Settele & Kuhn 2009).

*Maculinea* butterflies are obligate social parasites, since their larval survival depends both on the presence of specific food plants and specific *Myrmica* Latreille, 1804 ant species (Thomas 1980). After spending 10-15 days feeding on a species-specific food plant, *Maculinea* larvae drop to the ground and wait until they are found and carried into an ant nest by a *Myrmica* worker (Elmes et al. 1991a; Akino et al. 1999; Elmes et al. 2002; Thomas 2002). Adoption of the parasite caterpillars by the host ants is mediated by chemical deception (Akino et al. 1999; Schönrogge et al. 2004; Nash et al. 2008; Fürst et al. 2011). Once in the ant colony, butterfly larvae make use of different feeding strategies: *Maculinea alcon* and *M. rebeli* are called ‘cuckoo feeders’ because their larvae are fed directly by the ant workers by trophallaxis (Elmes et al. 1991b; Thomas & Elmes 1998), while *M. arion* and *M. teleius* are ‘predatory species’ and directly prey on ant brood. The alimentary strategy of *Maculinea nausithous* has not yet been fully clarified, but some authors suggest the coexistence of both “cuckoo” and “predatory” strategy or the predominance of the “cuckoo” behaviour (Thomas & Settele 2004; Patricelli et al. 2010). Irrespective of the species, *Maculinea* larvae spend 11 or 23 months inside their host colonies mimicking their host ants by both chemical and acoustical cues (Schönrogge et al. 2004; Barbero et al. 2009 a,b; Barbero et al. 2012; Witek et al. 2013).

In the past few decades, all *Maculinea* species have experienced severe declines over most of their ranges (Thomas 1995; Wynhoff 1998; Thomas & Settele 2004; Thomas et al. 2009). Consequently, they have attracted wide public attention, owing to their extraordinary life history and endangered status. At least partially as a consequence, *Maculinea* butterflies are mentioned in Annexes II & IV of the European Habitats Directive.

### **The *Maculinea alcon-rebeli* debate**

Among cuckoo species, *M.alcon* and *M.rebeli* are sometimes considered one of the best examples of ‘ecological races’ in butterflies, since they inhabit very distinct biotopes and show distinct ecological preferences (Descimon & Mallet 2009).

Historically, these two types have been considered either as distinct species, i.e. *Maculineaalcon* and *Maculinearebeli*, or as subspecies (or “Formenkreis”) of a single species (*M.alconalcon* and *M.alconrebeli*). Although their adult morphologies and genitalic characters are indistinguishable (Sibatani et al. 1994; Pech et al. 2004), their separation into two species has been accepted by many authors, mainly on ecological criteria (e.g. Munguira 1989; Thomas et al. 1989; Elmes et al. 1991a,b; Munguira & Martin 1999). The separation was made principally according to habitat characteristics, their initial larval food plant, and the host ant species. *M.alcon* occurs on wet meadows dominated by *Molinia coerulea* (L.) Moench, where females primarily oviposit on *Gentiana pneumonanthe* L., while *M.rebeli*’s main food plant is *Gentiana cruciata* L. and adults inhabit dry grasslands (Thomas 1995). Adaptation to different gentian species may also explain two important behavioural differences among populations of *M.alcon* and *M.rebeli*, i.e. the variation in caterpillars’ growth rate and in adult phenology. Sielezniew and Stankiewicz (2007) demonstrated that *M.rebeli* caterpillars using *G. cruciata* acquire about half of their final body mass before overwintering, while those of *M.alcon* adapted to *G. pneumonanthe*, gain most of their weight in the late spring of the following year. This is apparently tuned with the phenology of the two host plants. *G. cruciata* is in the appropriate phenological state for female oviposition a month earlier than *G. pneumonanthe*. As a consequence, *M.alcon* caterpillars have to accelerate their development after diapause to obtain the optimal timing of adult emergence (Sielezniew & Stankiewicz 2007).

All across their European distribution, *M.alcon* and *M.rebeli* populations use as hosts more than ten *Myrmica* species (see Thomas et al. 1989; Elmes et al. 1991a,b, 1994; Akino et al. 1999; Schlick-Steiner et al. 2004; Sielezniew & Stankiewicz 2004; Steiner et al. 2003; Tartally et al. 2008; Nowicki et al. 2009). Such a relatively large number of host switches, together with observations

that individual populations are typically highly species-specific with respect to ant association, suggest that cuckoos may be undergoing rapid ecological divergence (Elmes et al. 1994; Meyer-Hozak 2000; Als et al. 2001, 2004; Steiner et al. 2003; Witek et al. 2006).

Our findings support the existence of a clear separation of the two population groups. Italian populations of *M. rebeli* and *M. alcon* are characterized by marked phenological differences, being respectively on the wing from mid June till mid July, and from end July to end of August. Choices made by adult butterfly during oviposition provide even stronger evidences of sharp ecological separation between *M. alcon* and *M. rebeli*, as well as their use of host ant species (Czekes et al. 2013) (see the following paragraph).

Of course, the matter boils down to whether or not these two population types form separate clades. Even though some unpublished data obtained by K. Schönrogge and L. P. Casacci from larval epicuticular hydrocarbons would support their differentiation, other molecular studies based on sequence data of nuclear and mtDNA, or on allozymes (Als et al. 2004; Pech et al. 2004; Thomas & Settele 2004; Bereczki et al. 2005; Fric et al. 2007; Pecsénye et al. 2007), have failed to find evidence for a clade-level separation between *M. alcon* and *M. rebeli*. An important consequence of the unresolved taxonomic status of these two groups of populations is that the Appendixes and Annexes to the Bern Convention and to the EU Habitats Directive do not list them among species threatened of extinction in Europe (see also Kudrna et al. 2011). Habitat patches of both population types are becoming more and more isolated because of recent landscape fragmentation, generally due to natural forestation, owing to the abandonment of previous extensive agricultural practices and light grazing (van Swaay & Warren 1999). *M. alcon* is more severely threatened by the southern limits of its range, and perhaps especially in Italy, due to climate change and habitat degradation concomitant to the sinking water table. In north-east Europe, in contrast, *M. rebeli* is more vulnerable than *M. alcon* and needs urgent conservation actions, which should obviously be different from those for *M. alcon*.

A recent molecular investigation on 16 *M. alcon* and *M. rebeli* populations along ca. 700 km of the north-eastern edge of their distribution in Poland and Lithuania (Sielezniew et al. 2012) reopened the debate on the conservational status of these two taxa. As for previous studies, a sequence analysis of the nuclear EF1-a gene was insufficient for establishing an exact taxonomic classification of *M. alcon* and *M. rebeli*, but some microsatellite data were consistent with ecological host races. Combining EF1-a results and microsatellite information, the authors suggested the existence of at least three evolutionary significant units (ESUs) as defined by Crandall et al. (2000), corresponding to the north-eastern populations of *M. alcon* and the two geographically separated host races of *M. rebeli*, each of which would deserve specific conservation measures.

### **Host ants' diversification in *Maculinea* populations**

In the case of an obligate myrmecophilous species, the tight association with its host ant species may have forced selection for more locally adapted populations.

Since they inhabit ants' brood chambers and become highly integrated with their host society, cuckoo species show highly specific interactions with their host ants. They also receive frequent grooming and are fed by the nurse ants mainly by trophallaxis (cuckoo feeding). Individuals passing the initial period of integration usually survive well with any *Myrmica* ant species, so long as the colony remains well fed. However, if the colony experiences food shortages or any other similar stress, cuckoo species survive well only with their own specific host, while in non-host colonies, parasite larvae are killed (Elmes et al. 2004). The high level of host specificity is explained, therefore, by underlying integration mechanism.

On leaving its food plant, *M. rebeli* secretes a simple mixture of surface hydrocarbons that weakly mimic those of its host *Myrmica* ant, but it is sufficiently similar to all other *Myrmica* species for the larvae to be quickly retrieved by the first ant worker coming by. After adoption, the intruding larvae successfully integrate within colonies of the model host species, by synthesizing additional

hydrocarbons that more precisely mimic their *Myrmica* host (Schönrogge et al. 2004; Witek et al. 2013). By contrast, caterpillars adopted within nests of other *Myrmica* species suppress their secretions and rely on the passive acquisition of their current host colony odour (Schönrogge et al. 2004). Acquired camouflage alone, however, is an insufficient mechanism to survive periods of stress or deprivation inside the colony, when worker ants become more discriminating (Elmes et al. 2002).

The main cost of the cuckoo lifestyle is that increased specialization restricts each social parasite to a smaller, regional part of its host range. Thus, the host specificity pattern observed in *M. rebelie* and *M. alcon* is extremely complex, as a consequence of local adaptations. For instance, studies on *M. rebelie* from the Pyrenees show that its populations restrictively exploit colonies of *M. schencki* Emery, 1894 while eastern *M. rebelie* populations (mainly in Poland) use both *M. sabuleti* Meinert, 1861 and *M. scabrinodis* Nylander, 1846 (Thomas et al. 2005b, 2013). Thomas et al. (2013) have more recently suggested that this host shift could be a trace of a major difference in the chemical profiles, enabling each social parasite to infiltrate and exploit even very different *Myrmica* host societies. Extreme specialization makes each population incompatible for the survival with another's host species.

Analysing host specificity patterns in the Italian peninsula, we found that *Myrmica schencki* is the ant species most frequently used as “primary” host (sensu Thomas et al. 2005a) by all the *M. rebelie* populations investigated. In some cases, however, a shift towards ‘new’ *Myrmica* species was observed. Populations where the parasite is hanging in the balance between two host species may be interpreted as coevolutionary hot spots (Thompson 2005), where differentiation is in progress (Casacci & Barbero unpublished data; de Assis et al. 2012) (Figure 1).

[Insert Figure 1 about here]

A field study (Elmes et al. 2004) and an analysis of pre-adoption chemical profiles (Nash et al. 2008) suggested that similar differentiation may have evolved between the main European form of

*M. alcon*, which exploits *Myrmica scabrinodis*, and that of Scandinavia and the Netherlands, which is adapted to *Myrmica rubra*/*M. ruginodis*.

Ants association is therefore a double-edged sword for the conservation of these lycaenid butterflies, since it has promoted rapid rates of diversification, thereby creating a mosaic of overlapping ants and plant hosts, and has produced small, isolated non inter-exchangeable populations. This condition can lead to speciation, but if fragmentation increases in the face of anthropogenic disturbance and habitat loss, the risk of local extinction may also dramatically increase. The case of *Maculinea arion* in the United Kingdom remains emblematic: the species went extinct in 1979 because modest changes in grazing regimes and vegetation structure caused the host ant to be replaced by unsuitable congener species, unable to support the parasite's caterpillars (Thomas et al. 2009).

At molecular level, local host ant adaptations would be detectable only by markers linked with genes under selections by specific aspects of social parasitism. While waiting for geneticists to identify these markers, we are convinced that it is extremely important that myrmecophilous insect populations exploiting different host ants are recognised as separate Evolutionary Significant Units.

### ***Maculinea arion*: the genetic approach**

Among European *Maculinea*, the most remarkable decline was observed in *M. arion*. This species became extinct in the Netherlands in 1964 (Tax 1989), in the UK in 1979 (Thomas 1995) and in Belgium in 1996 (Goffart 1997; Thomas et al. 2009). It shows serious range contractions all over Europe, especially in its northern parts (Wynhoff 1998). As a consequence, the global status of *M. arion* has worsened during the last decade, from “near threatened” (van Swaay & Warren 1999) to “endangered” (van Swaay et al. 2010), following IUCN classification. Accordingly, *M. arion* is listed in Annex IV of the Habitat Directive. It is also considered an important indicator of habitat quality, as well as an umbrella species for several peculiar kinds of grassland communities (Randle

et al. 2005; Spitzer et al. 2009; Casacci et al. 2011). In other words, its protection provides indirect benefits to many other species.

The history of *M. arion*'s extinction in the UK demonstrates that a detailed ecological, demographic and genetic understanding of (threatened) species is crucial for a successful conservation strategy (Thomas et al. 2009).

The intraspecific taxonomy of the European populations of *M. arion* is confusing. Bereczki et al. (2011) have made a distinction between two ecotypes in the Carpathian region: (i) a first one, that these authors refer to *Maculinea arion arion* (Linnaeus, 1758), flying from mid-May to mid-June, prefers short-grass dry meadows and mostly uses *Thymus* spp. as initial food plant and (ii) another, which they tentatively refer to *Maculinea arion ligurica* (Wagner, 1904) (a taxon described thousands of kilometres away, in NW Italy) which is on the wing from the end of June to mid-August, mostly occurs at xerothermic areas and lays its eggs on *Origanum* flowerheads (Patricelli et al. 2011).

In the case of Carpathian populations, none of the criteria used to define ESUs is fulfilled: (i) the two groups of *M. arion* populations were not separated on the basis of allozyme studies and (ii) a certain extent of overlapping in their larval food plants was observed, thus weakening the idea of ecological isolation between the two forms.

Although the two forms generally have different habitat preferences, they often locally co-occur while remaining phenologically more or less isolated (Bereczki et al. 2011). Even though these authors envisaged that further studies are needed to enhance our understanding of the ecology of the two *M. arion* ecotypes, they argue that there are “non-evolutionary reasons” for attributing them conservation value (see Moritz 1994a,b). Even though Bereczki et al. (2011) concluded that what they called *M. a. arion* and *M. a. ligurica* cannot be considered separate ESUs according to more restrictive definition criteria, they attribute conservation value to both forms, because of their differentiation in phenology and habitat preferences.

We recently investigated the genetic structures of 20 *M. arion* populations from two distinct and geographically distant parts of Europe (i.e. Italy and Poland), which well-represent the ecological and morphological variation occurring across the European range of this species (Patricelli et al. 2013). The Polish populations occupy xerothermic grasslands occurring on southerly exposed slopes or sandy flat areas and exploit *Thymus* spp. as larval food plants. In Italy three morphoecotypes are present: in addition to the two already mentioned ones, *M. arion obscura* (Christoph, 1878), a dark coloured butterfly described from Zermatt and Stelvio, is found in the Alps where it colonises high altitude pastures and exploits *Thymus* spp. as host plant.

On the basis of the occurrence of putative subspecies and the fact that Italian populations were potentially able to survive glaciations, whereas Poland is a postglacial re-colonisation area, we were expecting to observe differences in the populations' genetic structures. Indeed, by sequencing the COI mitochondrial DNA gene (the 'barcoding gene') and the EF-1 alpha nuclear gene, we found substantial genetic differentiation among *M. arion* Italian populations in both markers, while almost no mtDNA polymorphism was found in the Polish samples.

More in detail, our analysis revealed that the Italian populations showed a high degree of polymorphism and divergence, highlighting their relevance in the context of biodiversity conservation, as well as from the evolutionary perspective. Rear Edge theory predicts that low-latitude populations of a species' distribution represent vital long-term stores of genetic diversity (Hampe & Petit 2005), which may partially explain differences in the observed genetic differentiation patterns. Our genetic data, however, did not support any subspecies-level divisions. Among our Italian samples were representatives of 'real' *M. a. ligurica*, *M. a. obscura* and *M. a. arion* (to which all the Polish populations supposedly belonged), which we all found genetically indistinguishable (Patricelli et al. 2013). Moreover, in agreement with Bereczki et al.'s (2011) allozyme studies, our results revealed no differences between *Origanum*- and *Thymus*-dependent populations. So, despite the ecological and morphological variability which led to the description of several subspecies, *M. arion*'s history may either have lacked events of deep population isolation,

or these events left no observable trace in the analysed genes. We speculated that the exploitation of different but related host plants by a locally monophagous butterfly species may represent recently evolved local adaptations. The persistent ecological and behavioural adaptations shown by local populations of *M. arion* as concerns habitat preferences and food plants exploitation, together with the genetic “southern richness” that we observed, however, are valuable enough criteria to consider some (single or multiple) Italian populations as separate Evolutionarily Significant Units.

## Conclusions

*ESUs, “crown” species vs. “basal” species, and “large” species vs. “small” species*

Many different choices can be made at the moment one has to decide where to allocate the normally sparse available economic resources. Vane-Wright et al. (1991) and Humphries et al. (1995) have suggested that priority should be recognised to species showing highest characters’ richness in phylogenetic reconstructions. In most cases, this means that species taking “basal” positions in cladograms may have highest conservation value. More in general and even though efforts to take phylogenetic diversity as a proxy for functional diversity, or evolutionary potential, have largely failed (Srivastava et al. 2012; Winter et al. 2013), choices will theoretically span between two extremes, such as either focusing on animals such as Coelacanths, which may have relatively scarcely evolved in the course of many millennia (Amemiya et al. 2013), or on some now dynamically evolving species, often found among the “crown” taxa, which may arguably represent the leading edge of evolution. The heuristic value, for conservation biology, of dealing with “small”, rather than “large”, taxonomic units has not yet been debated in sufficient depth and is probably far from being concluded (see Descimon & Mallet 2009; Frankham et al. 2012; Zachos et al. 2012; Groves 2013 and the literature cited therein for an excursus). It is worth noting that many international organisations implicitly or explicitly accept that units of conservation (ESUs?), which they generally treat as “species”, may be very restricted indeed (see Mittermeier et al. 2009, among many others). Even some very conservative authors (from this point of view), such as Frankham et

al. (2012, p. 30) seem to accept that “for allopatric [taxa]... adaptive differentiation among populations” may represent evidence for speciation. The notion that even individual populations of taxa having highly fragmented distribution may represent Conservation Significant Units (CSU) is therefore gaining some ground (see Yuan et al. 2011). In other words, declarations to the effect that we should concentrate our scarce resources to protect ONLY the most sharply defined taxa, are in sheer opposition to the ESU concept, which was devised to stimulate us to conserve all products of the current evolutionary processes, anywhere we happen to be able to reveal them, in terms of local adaptations. More in particular, we argue that limiting the field to those taxa that are grossly and obviously independent on morphological and/or molecular bases, would result in a wholesale sell out of the dynamics of our biodiversity. Apart from cases such as those we have already mentioned, we are making reference here in particular to taxa such as those of the *Euphydryas aurinia* (Rottemburg, 1775)/*E. provincialis* (Boisduval, [1828]) /*E. beckeri* (Lederer, 1853)/*E. glaciegenita* (Verity, 1928) complex, which are obviously adapted to completely different habitats and food-plants. Only the first of these, which occupies the European (and N. Italian) plains, is really threatened, while the rest of them are not threatened, at least for the time being.

### *Final Remarks*

As we have already mentioned in the beginning of this review, national and European legislations, at least for the moment, almost exclusively take into account species-rank taxa, so that the ESU concept has not yet broken much ground, in this respect. In contrast, however, all guidelines for the re-introduction of extinct populations of threatened species issued by international organisations (see IUCN 1998), or Conventions, make indirect reference to this notion.

Molecular studies of supposedly more or less severely threatened species have become increasingly common and the ESUs evaluation has become even more often based on evidence from the DNA and/or allozymes data only. We contend that this approach is reductionisticly mistaken. Despite that molecular approaches clearly represent a break-through in the rapid assessments of many

imperfectly known taxa, sometimes occurring in insufficiently explored areas, or for the discovery of cryptic biodiversity, a variety of sources, including ecological, behavioural, bio-geographic and morphological data, are equally valid for a strictly scientific assessment of conservation units. Of course, an important prerequisite for the use of non-genetic data consist in the assumption that observed traits are based on inheritable attributes, so that they can provide objective hierarchical information (Avice 1989; Waples 1991; Dizon et al. 1992; Moritz 1994a,b; Vogler & Desalle 1994; Lai & Pullin 2004). Convergence of information between molecular and non-molecular data may often provide clear evidence for ESU identification, but what conclusions can be drawn in those cases where information remains controversial or perhaps insufficient? Some species, such as *Coenonympha oedippus* (Fabricius, 1787), *Colias palaeno* (Linné, 1761) and *Lycaena dispar* ([Haworth], 1802) are still apparently prospering in the southern, Italian slopes of the Alps and relating plains, while they are endangered in several other parts of Europe (Cerrato 2013; Dolek et al. 2013), for still at least partially unclear reasons, since indications of genetic differentiation of the Italian populations are only and partially available for *L. dispar* (Lai & Pullin 2004). In other cases, biological differences such as those observed in *Euphydryas maturna* (Linné, 1758), seem to be spatially organised in a European mosaic of biological features, whose genetic basis remains, for the moment, uncertain (see Dolek et al. 2013).

Conversely, a survey of published genetic data on 31 European butterflies of conservation interest (Forister et al. 2008) has shown that the dilemma: “are observed genetic differences really adaptive?” may be more widespread than expected and stressed that results from molecular approaches should be used with caution, at least in conservation genetics. Frequent conflicts between data obtained from phylogenetic studies based on the DNA sequences of one or two loci and subjective (by definition) taxonomy, ultimately teach us that the diagnosis of conservation units should be based on the full recognition of the multiple forces capable of driving the evolution of molecular, ecological, morphological and behavioural characters (Rubinoff 2006). Non-molecular data provide critical information on differences between taxa recently originated by the action of

separate adaptive processes, which cannot generally be detected, at least in practice, by current DNA analyses. The combination of data from multiple genetic markers with the most traditional taxonomic approach, together with those from ecological and behavioural observations is the only effective way to avoid incorrect diagnoses. Observed discrepancies between these types of data highlight that many taxa that we should wish to conserve are of relatively recent origin. As a consequence, all processes at the population level should be considered when trying to identify conservation units, and surely not only the assumedly neutral dynamics that underlie mitochondrial or “bar-coding” level DNA evolution (Forister et al. 2008; see also Hickerson et al. 2006).

Finally, we wish to stress that biological conservation should not be based only on species prioritisations based on static parameters, such as those most easily perceived from the expression “we should try and preserve what we have for future generations”. While dealing with problems related to biological conservation, we should rather accommodate all those taxa apparently positioned on an “evolutionary front”, i.e. those characterised by recent (micro)speciation events and which may have the highest potentials for future biodiversity dynamics (see Erwin 1991).

Indeed it may be naïf to expect that these notions may directly and soon find a way into the European legislation. One could hypothesise, however, that EU Member States will be urged to include in a SCI not only as many populations as possible of each Annex II species, but also all the ESUs that may be directly or indirectly referred to each of them. Among other advantages, this would also create a legislative framework to cover the many ‘new’ taxa continuously separated as a consequence of ongoing DNA or ecological work.

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## Table

Table I. Evolutionarily Significant Unit (ESU) definitions since Ryder (1986) modified from de Guia & Saitoh 2007.

Authors	Definitions about ESU concept
<b>Basic concept</b>	
Ryder (1986)	Population units presenting significant adaptive variation based on concordance between sets of data derived by different techniques (life history information, morphometric, range and distribution data, and genetic data)
<b>Definitions stressing the importance of molecular data</b>	
Dizon et al. (1992)	Populations exhibiting discontinuous genetic divergence patterns, geographic isolation, and significant genetic distances
Avise (1994)	Sets of populations derived from consistently congruent gene phylogenies
Moritz (1994a,b)	Populations that are: (1) reciprocally monophyletic for mtDNA alleles; (2) demonstrate significant divergence of allele frequencies at nuclear loci
Bowen (1998)	Populations which show evidence of long-term isolation
Fraser & Bernatchez (2001)	A lineage demonstrating highly restricted gene flow from other such lineages within the higher organizational level of the species
<b>Definition stressing the importance of using molecular analysis and ecological data</b>	
Vogler & Desalle (1994)	Groups that are diagnosed by characters which cluster individuals or populations to the exclusion of other such clusters
Waples (1991)	A population or group of populations that: (1) is substantially reproductively isolated from other conspecific population units; (2) represents an important component of the evolutionary legacy of the species
Crandall et al. (2000)	Prefer identification of distinct populations characterized by genetic and ecological inexchangeabilities over recent and historical times
De Guia & Saitoh (2007)	Full ESUs can be defined solely when both information about neutral genetic variation and adaptive variation are available; otherwise the taxon are ascribed to partial ESU

## Figure Legend

Figure 1. Distribution of available *Myrmica* ant species and their use as primary or secondary hosts (*sensu* Thomas et al. 2005) by *Maculinea rebeli* at 5 selected Italian sites. The observed trend in host shifts along the Italian peninsula is shown in the rectangle on the upper right corner of this picture. **A** = *M. schencki*; **B** = *M. lobicornis*; **C** = *M. scabrinodis*; **D** = *M. sabuleti*; **E** = *M. lobulicornis*; **F** = *M. sulcinodis*; **G** = *M. ruginodis*.