16 • Bad species

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SUMMARY

Taxonomists often added the term *bona species* after the Linnaean binomial. The implication is that there are also *malae species*. A 'bad species' is a taxonomic unit that does not conform to criteria used to delimit species. The advent of numerical taxonomy and cladistics has upset earlier taxonomic certainty and two different consensuses seem to be building among evolutionary biologists. The species concept either (a) takes the form of a minimal, Darwinian, definition which ignores evolutionary mechanisms to allow universal applicability or (b) attempts to combine a variety of species concepts together. Under both views, species may evolve or be maintained via multiple different routes. Whenever there is conflict between criteria, or whenever regular hybridization occurs, in spite of the fact that the taxa remain to some extent morphologically, ecologically or genetically distinct, or if populations are allopatric but seem at that stage of divergence at which species fusion is doubtful, one may speak of 'bad species'. The tools used in making a decision on the rank of taxa at this stage of divergence include morphological, chromosomal (karyological), molecular, and ecological characters.

Two main groups of questions are addressed. Firstly, do species exist as real entities in nature, or are they a construct of the human desire for categorization and classification? Secondly, what are species made of, how do they arise and how are they maintained? And, are species a homogeneous rank from this evolutionary point of view?

Around 16% of the 440 European butterfly species are known to hybridize in the wild. About half or more of these hybrids are fertile, and show evidence of backcrossing. Detailed accounts are given for (a) the genus *Hipparchia*, (b) *Polyommatus* (*Agrodiaetus) admetus* and the 'anomalous blue' group, (c) the sibling species *Leptidea sinapis* and *L. reali* – with a comparison to the situation in *Melitaea athalia*, (d) *Zerynthia rumina* and *Z. polyxena*, (e) for the frequent hybridizations and introgressions in sympatric Papilionidae (*Papilio machaon* and *P. hospiton*; *Parnassius apollo* and *P. phoebus*), (f) for *Polyommatus* (*Lysandra) coridon, L. hispana* and *L. albicans* with frequent hybridization everywhere (with species remaining distinguishable), (g) for the *Erebia tyndarus* group, (h) for *Erebia serotina* (a hybrid mistaken for a species) and (i) for some briefly mentioned further examples.

There is justification for reviving the rather neglected (and misused) rank of subspecies, with the trend among lepidopterists to consider only more strongly distinct forms (in morphology, ecology or genetics) as subspecies, and to lump dubious geographical forms as synonyms. These recommendations provide a useful compromise between descriptions of geographical variation, the needs of modern butterfly taxonomy, and Darwin's pragmatic use of the term species in evolutionary studies.

It is a Sisyphean task to devise a definitive, irrefutable definition of species, but species will continue to function as useful tools in biology for a long time. Studies of gene exchange in the many hierarchical layers of phenotype, genotype and genome in 'bad' species of butterflies will illuminate the nature of speciation and evolution at the species level more than discussions on the 'essence' of species.

INTRODUCTION: SPECIES CONCEPTS AND TAXONOMIC PRACTICE

Taxonomists, when describing a new species, often added the term *bona species* after the Linnaean binomial. The implication is that there are also *malae species*. A 'bad species' is a taxonomic unit that misbehaves with respect to criteria used to delimit species. There are a wide array of species definitions linked to theories of speciation and evolution (Harrison 1998, Coyne & Orr 2004) and there have been many debates, which often become abstruse and epistemological (Wilson 1999a, Hey 2006). The biological species concept (BSC), based on reproductive isolation and associated with the theory of allopatric speciation, prevailed for many years. More recently, the advent of numerical taxonomy (Sokal & Crovello 1970) and cladistics (Hennig 1968)

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has upset the earlier certainty. The establishment of a basis for conceiving (Maynard-Smith 1966) and observing (Bush 1969) sympatric speciation led to suspicions that species were more indefinite, even locally, than architects of the modern synthesis had imagined. Today, two different consensuses seem to be building among evolutionary biologists. The species concept either takes the form of a minimal, Darwinian, definition which is agnostic about evolutionary mechanisms to allow universal applicability (Mallet 1995, Feder 1998, Jiggins & Mallet 2000), or attempts to combine a variety of species concepts together (de Queiroz 1998, Templeton 1998a, Coyne & Orr 2004). Under both views, species may evolve or be maintained via multiple different routes.

Species concepts and criteria: speciation theory and systematic practice

When treating an actual fauna or flora, the central problem is of the purely taxonomic criteria for species status. For a long time, four kinds of criteria have been used to group members of a species: character-based or 'syndiagnostic' criteria (which may use morphological or genetic traits); phylogenetic or 'synepigonic' criteria; reproductive, 'mixiological', or 'syngamy' criteria; and finally geographical criteria, particularly 'sympatry', 'cohabitation', or geographical overlap (Poulton 1904b; see also Jordan 1905, Rothschild & Jordan 1906, Cuénot 1936). To be distinct at the level of species, taxa should provide at least some of these four kinds of evidence. With the advent of the BSC (Dobzhansky 1937, Mayr 1942), the main emphasis was put on reproductive isolation (i.e. mixiological) criteria. This caused something of a divorce between evolutionary theory and taxonomic practice. Although an overwhelming amount of work has been carried out on the genetics and evolution of species – studies of genetic structure within species, interspecific crosses in the laboratory and field studies on hybrid zones (Barton & Hewitt 1989, Berlocher 1998, Coyne & Orr 2004) – practising taxonomists often continue to use syndiagnostic methods based mainly on morphological characters.

Indeed, when taxonomists have a sample of specimens coming from an unexplored geographical area, they can find morphological differences with taxa already described, but it is difficult to determine whether they are due to a few pleiotropic gene changes (i.e. the new samples are merely morphs of described taxa), to intraspecific geographical variation (subspecies), or to differentiation at full species level. Sometimes, rare hybrids between well-known species have even been mistaken for 'good' species. Since they are inaccessible, other criteria are simply ignored. Although they can reveal much about mixiological criteria, chromosomal and molecular characters are often used in much the same way as early taxonomists used morphological data; for instance, differences in chromosome numbers or the presence of diagnostic allozyme loci have been considered proof of distinct species, without consideration of geography or genetic relationships. We argue that these biological characteristics cannot be ignored.

Study of ecological niches is particularly important for associating morphological or genetic differences with different habitats (Sneath & Sokal 1973). Mayr, in later versions of his BSC (1982) argued that each species 'occupies a biological niche in nature'. Adaptive evolution is recognized as a primary means of both splitting and maintenance of separate lineages (Van Valen 1976, Templeton 1989, 1994, 1998a, Andersson 1990, Baum & Larson 1991, Schluter 2000). Sympatric speciation also involves ecological differentiation (Bush 1969, Feder 1998), and increasing evidence suggests that ecological divergence may directly cause reproductive isolation (Dodd 1989, Schluter 2001).

Nonetheless, mixiological criteria remain the most important within the BSC conceptual framework. They are reached through observation of the relations between the taxa either in sympatry, or in hybrid zones in the case of parapatry (O'Brien & Wolfluss 1991, Jiggins & Mallet 2000) – the latter are considered as 'natural laboratories for evolutionary studies' (Hewitt 1988) (see Chapter 19). Modelling as well as empirical studies suggest that hybrid zones can act as a barrier to gene flow (Barton & Hewitt 1989). Within them, the intensity of hybridization may vary. If hybrid genotypes predominate, the hybrid zone is considered 'unimodal', while, if genotypes are predominantly parental, with few intermediates, it appears phenotypically 'bimodal' (Harrison & Bogdanowicz 1997, Jiggins & Mallet 2000). Pairs of species that cohabit broadly and hybridize regularly can be studied genetically in the same way. In hybrid zones, the mixiological criterion of species depends on the fraction of genes that are actually exchanged between the taxa. Hybrids can be detected using morphological criteria, but this can be inaccurate, which makes it hard to estimate gene flow. Gene exchange, or introgression (Stebbins 1959), may transfer important genetic variation in some cases of adaptive evolution, especially in plants (Arnold 1992a, 1997, Mallet 2005). In birds and fish, hybridization is widespread (Grant & Grant 1992) and may be involved in rapid adaptive radiation and speciation (Grant & Grant

1998, Seehausen 2003). This also seems likely in *Heliconius* butterflies (Gilbert 2003, Bull *et al.* 2006). Introgression can affect the mitochondrial genome (Aubert & Solignac 1990) but, in Lepidoptera, where the Y-bearing sex is the female, Haldane's rule severely hinders mitochondrial introgression (see below and Sperling 1990, 1993, Aubert *et al.* 1997).

Based on the ideas of Mallet (1995) and Feder (1998), the separation of gene pools during speciation has been dubbed 'the genic view of speciation' by Wu (2001): speciation may not take place via separation of the whole gene pools, as postulated by the Dobzhansky–Mayr theory of speciation, but initially concerns only genes actively involved in reproductive isolation. The rest of the genome may still undergo sufficient gene flow to prevent differentiation, except in genomic regions tightly linked to 'speciation genes' (Ting *et al.* 2000). But what are speciation genes? Genes involved in divergent adaptation and mate choice should diverge first, and those causing hybrid sterility and inviability should be expected to diverge only after initial genetic separation. Complete separation should result from reinforcement of sexual isolation and further ecological differentiation (Noor 1999). Although Wu's genic view of speciation elicited an immediate rebuttal from the father of the BSC (Mayr 2001), it is clear that the proposed scheme is not that different from the 'classical' view of speciation according to Mayr. The most important distinction is that Wu's modification of Mayr's speciation scheme renders it compatible with a more substantial phase of gradual divergence in sympatry or parapatry.

An array of varied data obtained from difficult or 'bad' taxa can be used to support or refute the presence of additional species within a sample. The more concordant the data are, and the more bimodal the frequency distributions of phenotypes and genotypes, the more likely separate species status will be granted. These are methods termed 'genealogical concordance' or 'genotypic clustering' (Avise & Ball 1990, Mallet 1995). Similar syndiagnostic procedures were, in fact, being applied to morphological characters long before Darwinian times (Adanson 1763). As early as 1930, Nilsson (cited by Cuénot 1936) used the term 'genotypenkreis' to characterize species in *Salix*, a plant genus prone to hybridization.

This ideal procedure for species delimitation, careful study in zones of contact, is not always possible. In cases where concordance between criteria is imperfect, some argue for distinction at species level, and others against it. For instance, cryptic or sibling species (Dobzhansky 1937, Mayr 1963) fail to show diagnostic morphological characters; species that are otherwise well characterized apparently share the same ecological niche; hybrid zones can be unimodal in some areas and bimodal in other parts of the range. Molecular markers may be strongly differentiated among populations within species; in other groups, species clearly distinct using other criteria can show little molecular differentiation, especially if speciation is recent compared with the rate of molecular divergence.

Cohabitation: the lumper's species criterion adopted here

The touchstone of all criteria for separate, biological species is the test of 'cohabitation': whether overlapping populations produce unimodal (in which case subspecies might be designated), or bimodal (in the case of separate species) morphological and genotypic frequency distributions. This procedure dates from the late nineteenth century, and was promoted particularly vigorously for the Lepidoptera by Karl Jordan (e.g. Jordan & Rothschild 1906). Other species criteria that do not depend on degree of hybridization or intermediacy in areas of overlap are also in use today. In particular, Cracraft's (1983, 1989) 'phylogenetic' or 'diagnostic' concept is contributing to taxonomic inflation of 'species' numbers in birds, primates, and other taxa (Isaac *et al.* 2004), even when no new populations have been discovered. In butterflies, the prohibitive diversity of morphologically or genetically diagnosable local populations, usually referred to in our literature as 'subspecies', has tended to prevent such rampant splitting (for the moment). Here, we adopt this traditional and more inclusive, polytypic or 'lumper's' criterion for species.

When sympatric taxa hybridize very rarely, they can be classified as separate species. But what can be concluded if the units to be compared are not in contact? Breeding and crossing experiments provide an apparent solution, but this can be misleading. In particular, viability of hybrids in the laboratory may appear normal while, in nature, hybrids could be severely disadvantaged. Pre-mating barriers to hybridization can also be reduced under artificial conditions. In both cases, the degree of mixiological separation estimated can be spurious.

Whenever there is conflict between criteria, or whenever regular hybridization occurs, in spite of the fact that the taxa remain to some extent morphologically, ecologically or genetically distinct, or if populations are allopatric but seem at that stage of divergence at which species fusion is doubtful, one may speak of 'bad species'. The tools used in making a decision on the rank of taxa at this stage of divergence include morphological, chromosomal (karyological), molecular and ecological characters. In addition, one may cross such taxa, to obtain criteria relevant to reproductive isolation and introgression, keeping in mind the caveat previously invoked. These tools are described in detail in the appendix.

As with any term, 'species' must have a definition that depends partly on theoretical considerations. At this point, one might ask two main groups of questions: (1) Do species exist as real entities in nature? Or are they a construct of the human desire for categorization and classification? (2) What are species made of? How do they arise? How are they maintained? And are species a homogeneous rank from this evolutionary point of view? To answer such questions, it is necessary to investigate actual problem cases in some depth, which is the main aim of the rest of this chapter.

HOW COMMON ARE BAD SPECIES IN EUROPEAN BUTTERFLIES?

It is often said that, although there are disagreements about species concepts, there are few cases where our ability to delimit species is severely challenged (e.g. Mayr 1963). However, hybridization and bad species are rather more common than field guides tend to mention. Taxonomists overlook 'dubious' individuals (which may often be hybrids) because they make species discrimination more difficult. Natural hybridization occurs between around 10% of all animal species, although there are many groups where hybridization rates are greater (Mallet 2005). Here we provide collated data on European species, one of the best-studied faunas in the world (Table 16.1). Overall, around 16% of the 440 butterfly species are known to hybridize with at least one other species in the wild. Of these perhaps half or more are fertile, and show evidence of backcrossing in nature.

CASE STUDIES: THE PRACTICE OF EUROPEAN BUTTERFLY TAXONOMISTS AT SPECIES LEVEL

European butterflies are taxonomically well known. In the first comprehensive work on European butterflies, Higgins & Riley (1970) enumerated 371 species (including the Hesperioidea); in a recent book of the same scope, Tolman & Lewington (1997) record 440 species, 69 more. Amongst the 'new' European species, hardly any are actually new finds; many arise from 'taxonomic inflation', the upgrading of previously known subspecies to species level, or discoveries of known non-European species just inside the boundary (Dennis 1997, Isaac *et al.* 2004). In this section, we present an analysis of some decisions that illustrate how splitting and/or lumping has been performed in particular cases.

The genus *Hipparchia*: splitters and lumpers at work

Some genera have undergone especially intense splitting, like the graylings (*Hipparchia* and *Neohipparchia*). According to Higgins & Riley (1970), there were only 10 species in Europe. Today, there are 19 (Tolman & Lewington 1997), to which one more, *H. genava*, can be added according to Leraut (1990). Mostly, this proliferation is due to elevation to species rank of forms inhabiting islands or other disjunct geographic regions (e.g. *H. azorina*, *H. caldeirense* and *H. miguelensis* in the Azores). However, this is not true for *H. alcyone* and *H. genava*, between which Leraut records a hybrid zone. In a revision of the genus (Kudrna 1977) elevation to species rank was based only on morphology. Morphometric analyses of multiple, well-replicated samples in the *semele* group based on genitalia, wing-pattern measurements and allozyme electrophoresis were later carried out by Cesaroni *et al.* (1994), who showed convincing congruence between the morphometric analysis of genitalia and allozymes, although wing patterns followed an obviously different evolutionary pathway. The number of taxa with specific status was reduced by Cesaroni *et al.* from eight to five. As the taxa were largely allopatric and often insular in distribution, cohabitation and hybrid zone criteria cannot be tested. Assignment to species level was therefore performed on the basis of 'sufficient' genetic distance (Nei's D between 0.07 and 0.26).

Later, Jutzeler *et al.* (1997) presented another treatment of the same group. Although devoted mainly to meticulous morphological description of certain taxa and their first instars, and lavishly illustrated with scanning electron microscope (SEM) pictures and excellent colour plates, the specific status of the various taxa was also discussed. The authors, it turns out, are extreme 'splitters', and even cite Cesaroni *et al.* (1994) to justify splitting – in complete contradiction to that paper. No morphometric analyses were performed while making these controversial decisions. More recently, even more 'insular splitting' has been carried out by Jutzeler *et al.* (2003a, b): taxa from the Tyrrhenian Islands were raised to species on the basis of morphological

Table 16.1A *Some examples of bad species in European butterflies, including all known records of interspecific hybridization in the wild*

Species 1	Species 2	Location	Hybrid frequency[a]	Characters, except morphology[b]	Taxonomic interpretation	Source
Papilio machaon	*P. hospiton*	Corsica, Sardinia	F	D, A, M, E, H(I), P!	Sibling species	See text
Iphiclides p. podalirius	*I. p. feisthamelii*	Languedoc	F	A, M	Parapatric sibling species	See text
Zerynthia polyxena	*Z. rumina*	Provence	R/E	P, G, C, A, M, E, H! I! S!	Parapatric species	See text, Plate 20b
Parnassius apollo	*P. phoebus*	Throughout the Alps	F	G, A, M, H(S)	Partially sympatric species	See text, Plate 19b
Artogeia napi	*A. bryoniae*	Alps	C/F	C,E,H^{-1}(I)	Parapatric sibling species	Bowden, 1996; Geiger & Shapiro, 1992; Porter & Geiger 1995
Artogeia napi	*A. balcana*	Balkans	?		?Parapatric subspecies	Tolman & Lewington, 1997
Artogeia napi	*A. rapae*	Britain, Germany	E	A	Sympatric species	Klemann, 1930; Heslop-Harrison, 1951
Pontia daplidice	*P. edusa*	Coastal S. France, Italy	F	A, H^{0}(I),G	Parapatric sibling species (narrow overlap)	Geiger *et al.*, 1988 ["semispecies"]; Wenger *et al.*, 1993 ["semispecies"]; Porter *et al.*, 1997 [regard as subspecies]
Euchloe crameri	*E. simplonia*	Alps, Pyrénées	Likely!	D, A Allozymes differ markedly (Geiger, pers. comm. to HD)	Parapatric, ecologically divergent forms	Lux, 1987; Descimon, unpub.
Anthocaris belia euphenoides	*A. cardamines*	S. France, Spain	R/E	H	Partially sympatric species	Legras in G&D, Plate 19c
Anthocaris damone	*A. gruneri*	Greece	E	A	Partially sympatric species	Rougeot, 1977
Colias crocea	*C. erate*	Greece, C. Europe	R/F		Partially sympatric species	Alberti, 1943
Colias hyale	*C. erate*	C. Europe	R	A	Partially sympatric species	Alberti, 1943
Colias crocea	*C. hyale*	Only in lab But likely to occur	E	Confused with aberrant *crocea*?	Sympatric species	Ryszka, 1949 5–7; Descimon in G&D
Colias myrmidone	*C. hyale*	E. Europe	E	A, M	Partially sympatric species	Mecke, 1923
Colias crocea	*C. phicomone*	Alps	R	E	Partially sympatric species	Descimon in G&D
Colias palaeno	*C. phicomone*	Alps	E	E	Partially sympatric species	Descimon in G&D G. Poluzzi, *fide* HD
Colias hecla	*C. tyche (= nastes)*	Norway, Sweden	F/R	"christiernsonni" Lampa	Sympatric species	Kaisila, 1950

Table 16.1A (*cont.*)

Species 1	Species 2	Location	Hybrid frequency^a	Characters, except morphology^b	Taxonomic interpretation	Source
Gonepteryx rhamni	*G. cleopatra*	S. Europe	E	E	Partially sympatric species	G&D; Descimon, unpub.
Leptidea sinapis	*L. reali*	Europe	None known	W−, P, G, A, M, E	Partially sympatric species	
Lycaena tityrus subalpina	*Lycaena hippothoe*	French Alps	R	E!	Partially sympatric species	Descimon in G&D Bernardi, pers. comm. to HD
Lycaena tityrus tityrus	*Lycaena t. subalpina*		C	E, H(I)	Parapatric strong ssp.	Higgins & Riley, 1970; Descimon, 1980
Cupido minimus	*E. alcetas*	W. France	E		Sympatric species	D' Aldin, 1929
Aricia agestis	*A. artaxerxes*	UK, possible elsewhere	E (ancient)	A, M, D, H(I)	Narrowly overlapping sympatric species	Wynne & Mallet, unpub.
Agrodiaetus damon	*A. rippartii*	Balkans	R	A, M	Partially sympatric species	Schurian & Hoffmann, 1975
Agrodiaetus damon	*Polyommatus meleager*	Alps	E	C, A, M, E	Intergeneric hybrid	Rebel, 1920
Agrodiaetus damon	*Polyommatus icarus*	Alps	E	A, M	Intergeneric hybrid	Rebel, 1930b.
Lysandra coridon	*L. bellargus*	Europe	F	C,G,D, A *polonus* Zeller	Sympatric spp.	See text, Plate 19a
Lysandra hispana	*L. bellargus*	S. France, Spain, Italy	R (rarer than *polonus*)	C, A, M =*samsoni* Verity?	Sympatric sp.	Cameron-Curry *et al.*, 1980
Lysandra bellargus	*L. albicans*	S. W. Spain	R	D, C, A, M	Distant species	Gómez Bustillo & Fernandez-Rubio, 1974
Lysandra coridon caelestissima	*L. albicans*	Central Spain	F	C, A, M *caerulescens* Vty	Partially sympatric species	See text, Plate 19a
Lysandra coridon	*Agrodiaetus damon*	Alps	E	G, C, A, M	Distant species	Rebel, 1930a; Descimon, unpublished
Lysandra coridon	*Meleageria daphnis*	Alps	R	C, A, M *cormion* Nabokov	Distant species	See text, Plate 19a
Lysandra albicans	*Plebicula escheri*	Spain	E	C, A, M, E	Intergeneric	De Carpentrie, 1977
Lysandra coridon	*Polyommatus icarus*	Germany	E	C, A, M, E	Intergeneric	Herrmann, 1926
Lysandra coridon	*Plebicula dorylas*	France	E	C, A, M, E	Intergeneric	Goodman *et al.*, 1925

Plebicula dorylas	*Plebicula nivescens*	Central Spain	R	C, A, M *caeruleonivescens* Verity	Partially sympatric related species	Verity in G&D; Descimon, unpub., Plate 19a
Polyommatus icarus	*P. eros*	Alps	E	A, M	Related species, sympatric on mountains	Descimon, unpub.
Polyommatus icarus	*Plebejus argus*	Germany	E	A, M, E	Intergeneric hybrid	Peter, 1928 140
Maculinea alcon	*M. rebeli*	All Europe	?	M, E	Good species or ecological races?	Wynhoff, 1998
Boloria pales	*B. napaea*	French Alps	F/R?	W–, G, A, M	Partially sympatric species	Descimon, unpub.
Euphydryas aurinia	*E. desfontainii*	Spain	R	G, A, M, E, HS	Partially sympatric species	De Lajonquière, 1966
Mellicta athalia athalia	*M. athalia celadussa*	Central France [?]	C	G, W–	Parapatric subspecies?	See text
Mellicta athalia	*M. deione*	Provence	E	G, A, M	Partially sympatric	Descimon, unpub.
Mellicta parthenoides	*M. varia*	Southern French Alps	F/R	C, H(I)!	Parapatric sibling species	Bernardi, pers. comm.; G&D
Melanargia russiae	*M. lachesis*	Eastern Pyrenees	E	A	Species	Tavoillot, 1967
Melanargia galathea	*M. lachesis*	France and Spain	F (only in some overlaps)		Parapatric sibling species	Higgins, 1969; Wagener, 1984; Essayan, 1990
Hipparchia semele	*H. (senthes?) balletoi*	Italy	R	G, A	Parapatric sibling species	Sbordoni, pers. comm.; but see text
Erebia flavofasciata	*E. epiphron*	Alps	R	G	Partially sympatric species	See text
Erebia pharte	*E. epiphron*	Alps	E	G	Sympatric species	Descimon in G&D
Erebia pronoe	*E. epiphron*	Pyrenees	R	H(I) =“*serotina*” Descimon & de Lesse	Sympatric species	See text, Plate 20a
Erebia pronoe	*E. medusa*	Carpathians	E		Distant species	See text
Erebia cassioides	*E. hispania*	Pyrenees	R, several zones in the Pyrenees	G, C, A, M	Parapatric sibling species	See text, Plate 20a
Erebia cassioides	*E. tyndarus*	Alps	F	A, M	Parapatric sibling species	See text
Erebia cassioides	*E. nivalis*	Alps	F	A, M, E	Parapatric sibling species	See text
Coenonympha arcania	*C. hero*	N. Europe	F *hero* nearly extinct		Partially sympatric species	Legras in G&D Gross, 1957
Coenonympha darwiniana	*C. gardetta*	Alps	F	*A. darwiniana* may be hybrid *gardetta* × *arcania*	Parapatric species	See text

Table 16.1B *Bad species supplementary data. Excluded from above because too doubtful or not studied enough; includes also some doubtful species/subspecies (these are included only if there is some cohabitation)*

Species 1	Species 2	Location	Hybrid frequency[a]	Characters, except morphology[b]	Taxonomic interpretation	Source
Pieris ergane	*P. napi*	S. Europe	L	W+/−, Lab	Partially sympatric species	Bred by Lorkovic
Euchloe crameri	*E. simplonia*	Maritime Alps	E to F? Not studied!	D, W+/−, A, E	Parapatric species (montane vs. lowland)	Lux, 1987
Euchloe simplonia	*Anthocaris cardamines*	Alps and Pyrenees	? (E)	A, HI, HS, Lab	Sympatric species	Obtained until pupa by HD
Lycaeides idas	*L. argyrognomon*	Central France	?, L+	W+/−	Sympatric species	HD's observations in Yonne
Lycaeides idas	*L. idas calliopis* Boisduval	S. French Alps	?	E, ?	Could be sibling species	Numerous observations since Boisduval, including HD's
Everes argiades	*E. alcetas*	S. Europe	L	W+/−	Partially sympatric	
Everes argiades	*E. decoloratus*	S. Europe	L	W+/− or W−	Partially sympatric	
Everes alcetas	*E. decoloratus*	S. Europe	L	W+/−	Partially sympatric	
Cupido lorquinii	*C. carswelli*	S. Spain	L	W+/−, E	Partially sympatric	
Glaucopsyche alexis	*G. melanops*	S. W. Europe	L	W+/−	Partially sympatric	Possibly captured by HD
Maculinea teleius	*M. nausithous*	Europe	?		Partially sympatric	
Pseudophilotes baton	*P. panoptes*	Spain	?	W−	May be subspecies or parapatric sibling species	
Pseudophilotes baton	*P. abencerragus*	S. Spain	L	W+/−	Partially sympatric	
Aricia agestis	*A. morronensis*	Spain		W−	Partially sympatric	
Agriades glandon	*A. pyrenaica*	Pyrenees	?		Partially sympatric	
Agrodiaetus rippartii	*A. fabressei*	Central Spain		W−	Partially sympatric	Should be very difficult to detect
Agrodiaetus dolus	*A. damon*	S. Europe	L (E)		Partially sympatric	As likely as *L. coridon* × *A. damon*!
Agrodiaetus dolus	*Agrodiaetus* (brown sp.)	S. Europe	L (E)		Partially sympatric	As likely as *A. dolus* × *A. rippartii*!
Polyommatus icarus	*P. eroides*	S. E. Europe	L		Partially sympatric	
Polyommatus eros	*P. eroides*	S. E. Europe	L	W+/−	Partially sympatric	
Polyommatus icarus	*P. andronicus*	Greece		G+	Partially sympatric sibling species	

Apatura ilia	*A. metis*	S. E. Europe	L	W−E−	Parapatric species	
Argynnis adippe	*A. niobe*	Palaearctic		W+/−	Widespread species	Why not?
Brenthis hecate	*B. daphne*	W. Palaearctic		E, W+/−	Partially sympatric	The three *Brenthis* often fly in close vicinity, in spite of marked ecological differences.
Brenthis hecate	*B. ino*	W. Palaearctic		E, W+/−	Partially sympatric	
Brenthis daphne	*B. ino*	W. Palaearctic		E, W+/−	Partially sympatric	
Clossiana selene	*C. euphrosyne*	W. Palaearctic		E, W+/−	Partially sympatric	
Circumpolar *Clossiana*	(5 species)	Scandinavia		W+/−	Partially sympatric	
Melitaea parthenoides	*M. aurelia*	Europe	L	W+/−	Partially sympatric	Suspected to occur in Briançon region (French Southern Alps) by HD
Melitaea aurelia	*M. britomartis*	Central Europe	?	W−, G+	Partially sympatric	
Melitaea phoebe	*M. aetherie*	S. Spain		W+/−	Partially sympatric	
Melanargia occitanica	*M. galathea* or *lachesis*	S. Europe			Partially sympatric	
Melanargia occitanica	*M. russiae*	S. Europe		E	Partially sympatric	
Melanargia occitanica	*M. ines*	Spain			Partially sympatric	
Hipparchia fagi	*H. alcyone*	S. Europe	L	W−	Partially sympatric	
Hipparchia sp.				W+/−	Partially sympatric	Several opportunities within this complex genus
Chazara briseis	*C. prieuri*	Spain			Partially sympatric	
Pseudochazara sp.		S. E. Europe			Partially sympatric	Cf. *Hipparchia*
Satyrus actaea	*S. ferula*	S. Europe		W+/−	Partially sympatric	Searched for by HD in Briançon region – in vain!
Erebia ligea	*E. euryale*	European mountains	L		Partially sympatric	Possibly found by HD
Erebia pharte	*E. melampus*	Alps	L	W+/−	Partially sympatric	Possibly found by HD
Erebia aethiopella	*E. mnestra*	French Alps	F	W+/−	Parapatric species	Bimodal hybrid zone at Montgenèvre, French Southern Alps (HD's and Claude Herbulot's observations)
Erebia stirius	*E. styx*	Central Alps		W−	Partially sympatric	Cf. Lorković's works; could also hybridize with *E. montana*
Hyponephele lycaon	*H. lupina*	S. Europe		W+/−	Partially sympatric	
Aphantopus hyperanthus	*Maniola jurtina*	Palaearctic			Largely sympatric	Pairing rather often observed, hybrids never

Table 16.1B (*cont.*)

Species 1	Species 2	Location	Hybrid frequency[a]	Characters, except morphology[b]	Taxonomic interpretation	Source
Pyronia tithonus	*P. bathseba*	S. Europe		W+/−	Oceanic vs. Mediterranean	
Coenonympha sp.						Several candidates in the genus in addition to those observed
Lasiommata maera	*L. megera*	Europe		W+/−	Largely sympatric	Suspected around Marseilles by HD
Lasiommata maera and *megera*	*L. petropolitana*	Alps, Pyrenees		W+/−	Sympatric in Alps and Pyrenees	

[a] Hybrid frequency: C, Common (Hardy–Weinberg); F, Frequent >1%; R, Regular <1%; E, Exceptional <0.1%; L, Likely, but no data.

[b] Characters enabling detection of hybridization, apart from wing pattern (− means does NOT occur): W−, No wing pattern differences; W+/−, differences not striking enough to allow recognition without especial attention; P, Mate choice differences; D, Diapause; G, Genitalia; C, Chromosomes; A, Allozymes; M, Molecular (nuclear and mitochondrial DNA); E, Ecological; H, Haldane's rule; H^{-1}, Inverse Haldane's rule; H^0, Non-Haldane rule inviability; I, Inviability (e.g. H^{-1}(I)); S, Sterility; Lab, hybrids have been obtained in captivity.

[c] G&D, Guillaumin & Descimon, 1976.

and bionomic differences with continental relatives, again without any morphometric, karyological, mixiological or molecular justification. Most of these 'new' species are allopatric. We tend to side with the more conservative views of Cesaroni *et al.* (1994).

Polyommatus (*Agrodiaetus*) *admetus* and the 'anomalous blue' group: chromosome variation and allopatry

According to Lukhtanov *et al.* (2003), 'this complex is a real stumbling block in the taxonomy of the genus [*Agrodiaetus*]'. In a careful study using the 'classical' tools of typological taxonomy, Forster (1956) was uncertain about the taxonomic status of only a few forms or 'bad species'. Soon thereafter, de Lesse (1960a) used karyology to show that the picture was not simple but death prevented him from carrying his work further. The *admetus* group of *Agrodiaetus*, which included only three species in Higgins & Riley (1970), was raised to nine some 35 years later (Tolman & Lewington 1997, Wiemers 2003).

In *Agrodiaetus*, the males are generally blue, but the 'anomalous blues' all have similar, chocolate-brown uppersides in both sexes. In 1970, the species recognized were *A. admetus*, ranging from Eastern Europe to Asia Minor, *A. fabressei* known only from Spain and *A. ripartii* from scattered locations from Spain to Asia Minor. This treatment was supported by karyotyping: $n = 78$–80 for *admetus*, $n = 90$ with two large unequal chromosomes for *ripartii* and $n = 90$ with two large and two medium-sized chromosomes for *fabressei* (de Lesse 1960a). The taxa *fabressei* and *ripartii* cohabited without admixture in some Spanish localities (de Lesse 1961a).

The situation became more complex when wide karyotypic variation was found in Turkey and later in parts of Europe (Table 16.2).

More recently, allozyme studies have cast doubt on this multiplicity of species. *Agrodiaetus ripartii*, the most

Table 16.2 *Variation in chromosome number of described species within the subgenus* Agrodiaetus

Species of *Agrodiaetus* (according to Tolman & Lewington 1997, Wiemers 2003)[a]	Distribution	Chromosome number (*n*)
admetus Esper	Bulgaria	80
admetus Esper	Turkey	78–80
alcestis Zerny	Lebanon	20–21
aroaniensis Brown	Peloponnese	48
dantchenkoi Lukhtanov *et al.*	Turkey	42
demavendi Pfeffer	Iran, Turkey	68–71
eriwanensis Forster	Armenia	32–34
fabressei Oberthür	Spain	90 (86+2+2)
galloi Balletto & Toso	S. Italy	66
humedasae Toso & Balletto	N. Italy	38
interjectus de Lesse	Turkey	29–32
karacetinae Lukhtanov & Dantchenko	Turkey	19
nephohiptamenos Brown & Coutsis	N. Greece	8–11, or ~90[b]
ripartii Freyer	Spain–Turkey	90 (88 + 1 + 1)

[a] Taxa with no information on chromosome number are omitted, as are taxa of obviously subspecific rank.

[b] There are contradictory numbers counted by Brown & Coutsis (1978) and de Prins (unpublished); the $n = 90$ estimate seems most likely (Wiemers 2003).

Source: From Hesselbarth *et al.* (1995), Eckweiler & Häuser (1997), Häuser & Eckweiler (1997), Carbonell (2001), Lukhtanov & Dantchenko (2002a, b, 2003), Wiemers (2003) and Kandul *et al.* (2004).

widespread, proved as homogeneous genetically as in its karyotype; this is also true, to a lesser degree, for *A. admetus*. *Agrodiaetus fabressei* and the other taxa are poorly resolved and there is little correlation between allozymes and karyotype (Mensi *et al.* 1994). More recently, mitochondrial and nuclear DNA sequencing studies suggest that 'brown' *Agrodiaetus* are polyphyletic. The wing colour switch from the 'primitive' blue colour to brown in males seems to have occurred twice: once in the '*admetus*' group and once in *fabressei* (Wiemers 2003, Kandul *et al.* 2004). Most distinguishable entities are allopatric, and the only exceptions are the aforementioned *A. fabressei* and *A. ripartii*, and four species found close together in the Turkish Van province (Lukhtanov *et al.* 2003). In most other cases, nobody knows what would occur if these genetic entities flew together.

Clues are provided by the *fabressei–ripartii* case, which have the same chromosome number, but differ in details of the karyotype. They comply with the cohabitation criterion and are genetically distant (Lattes *et al.* 1994). Clearly, there is little doubt that these are good (albeit sibling) species. However, they are almost impossible to identify using morphology where they co-occur, since neither wing pattern nor skeletal morphology provide reliable criteria: karyotype and DNA sequencing are virtually the only ways to assure identification (Lukhtanov *et al.* 2003). Chromosomal information has also been used by Munguira *et al.* (1994), who merged the Spanish *agenjoi* Forster and *violetae* Gomez-Bustillo *et al.* into the known species: *fabressei* and *ripartii*. However, Gil-T & Gil-Uceda (2005) showed that these authors did not examine the 'true' *violetae* (rediscovered after more than 20 years) from Sierra de Almijara (its type locality), but populations coming from ca. 200 km to the northeast (Sierra de Cazorla). Both populations are morphologically well differentiated. New karyological and biochemical studies hopefully will determine its final taxonomic status (Lukhtanov *et al.* 2005).

Chromosome structure is unstable in *Agrodiaetus* and rearrangements are common even within populations, leading to the formation of multivalents during meiosis (Lukhtanov & Dantchenko 2002a, b, Lukhtanov *et al.* 2003). Limited abnormalities seem not to affect viability, although selection should eventually eliminate most rearrangement polymorphism. Why is chromosome structure so unstable in *Agrodiaetus*? Kandul *et al.* (2004) argue that tolerance of chromosomal polymorphism is related to centromere structure, and suggest that destabilization of chromosome numbers may be due to locally abundant transposons. In allopatric populations of *Agrodiaetus*, elimination of differences will not take place and the karyotype diverges rapidly until a point of no return is reached, giving rise to a great deal of geographical variation, and ultimately speciation. Similarly, Wiemers (2003) boldly states that 'changes in the number of chromosomes do not lead to sympatric speciation, but instead appear as a by-product of allopatric speciation and such young species could only occur in sympatry after a sufficient differentiation in their phenotype to exclude erroneous matings'.

Leptidea sinapis and *L. reali*: sibling species and the almost 'perfect crime', with a comparison to the situation in *Melitaea athalia*

Until the end of the twentieth century, nobody suspected that two separate species lurked within the wood white, *Leptidea sinapis*. In 1962, Réal noticed that two different seasonal forms flew together in the French eastern Pyrénées, without considering the possibility that two species were involved (Réal 1962). By the late 1980s, after morphological studies on the genitalia, Lorković suggested to Réal that there were indeed two species. The latter described a new species under the name *lorkovicii* in 1988, an invalid name replaced by *reali* (Reissinger 1989). Further study confirmed that the two forms, characterized by male and female genitalia, were distinguishable and sympatric across much of Europe (Lorković 1994, Mazel & Leestmans 1996); in particular, the penis is short in *sinapis*, and long in *reali*. There are correlated differences in the females, with short vs. long ductus bursae. This strongly suggests a 'lock and key' mechanism is involved. Although other barriers may be present, it seems likely that these differences can explain reproductive isolation between the taxa. In contrast, earlier attempts to find reliable differences in wing pattern and ecology were in vain. *Leptidea sinapis* is present everywhere in Western Europe, while *reali*, if present, is always in sympatry with it.

Although the existence of two 'good' species is likely, it could be argued that there is merely a genitalic polymorphism, similar to that in *Melitaea athalia* and *M. celadussa* (see below). To address this point, a study based on multivariate morphometrics of genitalia, allozymes and mtDNA sequencing was undertaken by Martin *et al.* (2003) on six populations from southern France. A 728-bp fragment of the *ND1* gene showed a reliable and constant 3% divergence between the entities. Among 16 enzyme loci, none was completely diagnostic, but *Ak* and *Pgi* showed highly significant differentiation. Multivariate analysis demonstrated two well-separated 'genotypic clusters', with strong linkage disequilibria between loci. Furthermore, allozymes and the mtDNA were concordant. Morphometrics carried out on genitalia also yielded good concordance with molecular data, although there was some (<5%) overlap between the taxa. In 163 individuals of the two species, no hybrid was detected; the few individuals with doubtful genitalic measurements were clearly assigned to one or other taxon by molecular markers.

The necessity of dissecting individuals for identification makes ecological study difficult, and it was at first thought that the species fly together and share most foodplants. This should contradict Gause's principle but could explain the lack of consistent differences in wing pattern. However, the population genetic structure of the two species is somewhat different: *L. reali* is less polymorphic at allozymes (with heterozygosity $0.09 < H < 0.14$ in *sinapis* and $0.05 < H < 0.07$ in *reali*: Martin *et al.* 2003). Females and, to a lesser extent, males of both species discriminate between the species during mate choice, and only intraspecific matings occurred in captivity (Freese & Fiedler 2002). The two species are now known to differ in ecology: *L. sinapis* is a widespread generalist on various herbaceous Leguminosae from both wet and dry habitats, while *L. reali* specializes on *Lathyrus pratensis*, a plant confined to moist grasslands. In 347 localities in the Drôme department (southern France) where *L. sinapis* and/or *L. reali* were observed, *L. sinapis* was alone in 55% of the study sites, and *L. reali* in 22%, whereas both species were found together in 23% of them (Amiet 2004). There are also differences in phenology, response to temperature and habitat choice (Friberg *et al.* 2008). The situation seems to reverse in Eastern Europe, where *L. sinapis* becomes confined to warmer areas (Benes *et al.* 2003b). Freese & Fiedler (2002), in their mainly laboratory-based study, concluded that 'the two species are only weakly differentiated in ecological terms'; indeed, their egg-laying tests showed only a weak preference for *L. pratensis* in the females of *L. reali*; the larvae of both species prefer and perform better on another legume, *Lotus corniculatus*, a result rather discrepant with Amiet's (2004) field observations.

As in almost all 'perfect crimes', once the first clue was discovered, a cascade of confirmatory data was quickly revealed. At the end of the nineteenth century, the earliest dissectors of genitalia, such as Reverdin, could well have studied a series of *Leptidea* male genitalia and discovered the two species.

The latter did just this with *Melitaea athalia* (Reverdin 1920, 1922), where two types of male genitalia were associated with two biogeographical entities, and he therefore split them into separate species. However, later study showed that the morphology of male genitalia was unimodal within a hybrid zone between the two taxa. The width of the hybrid zone varied from a few to several tens of kilometres (Bourgogne 1953). Since this differentiation is not associated with large and constant differences in allozymes or mtDNA, as in *Leptidea* (Zimmermann, unpublished), species separation in *Melitaea* was premature.

Zerynthia rumina and *Z. polyxena*: relativity of mixiological criteria

The genus *Zerynthia* contains two species, both recognized since the dawn of entomology: *Z. rumina*, a western Mediterranean species, and *Z. polyxena* from the eastern Mediterranean (Plate 20b). They overlap in southern France, where they display marked ecological differentiation, while in areas where only one species is found, both have a more extensive niche. Besides wing-pattern differences, there are diagnostic alleles between, with Nei's $D \approx 0.80$ (Braconnot, unpublished) and strong divergence in mitochondrial and nuclear gene sequences (Nazari *et al.* 2007). There is no doubt they are 'good' species. Both display marked intraspecific differentiation: wing patterns of the French subspecies *Z. rumina medesicaste* and *Z. polyxena cassandra* clearly differ from their respective nominal subspecies, but variation forms a wide cline within a continuous distribution.

Natural hybrids between the species are scarce (only five are known to HD), but interspecific pairing has been observed in the field (de Puységur 1947). A large series of crosses within and between species has been performed by HD, although only some have been published (Descimon & Michel 1989). When *Z. rumina medesicaste* was crossed with *Z. r. rumina*, remarkable hybrid vigour was observed in the F_1, followed by strong hybrid breakdown in the F_2 (i.e. $F_1 \times F_1$) with arrested embryonic development, larval weakness and difficulties of pupation. Fewer than 5% of ova reached the adult stage in about 10 parallel broods. The low viability persisted in further crosses; only backcrosses, with either parent subspecies (or, paradoxically, with *Z. polyxena*), restored viability. Crosses between *Z. p. polyxena* from Greece and *Z. p. cassandra* from southern France also produced F_1 hybrid vigour, and some F_2 hybrid breakdown. However, the F_2 viability was not too low (around 25%), and further crosses ($F_2 \times F_2$ and more) displayed markedly enhanced viability: incompatibility therefore seemed less marked than in the first case. Crosses between Austrian and French *Z. polyxena* produced no F_2 hybrid breakdown.

Mate choice was studied in cages containing 10 males and 10 females of each species. Only intraspecific matings were observed (including the aforementioned distinct subspecies), demonstrating strong prezygotic barriers between species. All females proved to have mated, and one female *polyxena* produced offspring consisting partly of *polyxena* and partly of hybrids. Clearly, she had mated twice, and with males of each species. The hybrids were viable, but while the F_2 resulted in no offspring, backcrosses with *polyxena* and *rumina* were successful. The backcross hybrids from either side could, however, be crossed with the more distant parental strains. Thus backcrossed individuals, which had 3/4 of their genes from one species and 1/4 from the other, gave symmetrical F_3 progeny with 3/8 *rumina* : 5/8 *polyxena* offspring and the reciprocal; the same scheme was applied in the F_4 and beyond. The possibilities for complex crosses increased with the rank of hybridization and some were practised (for a complete account, see Descimon & Michel 1989). The hybrids were viable provided they had at least one complete unrecombined genome from a parental strain. Much more surprisingly, two later hybrid × hybrid crosses (not many were tried) gave fairly viable offspring, with no significant departures from 1 : 1 sex ratio or diapause abnormalities. In spite of strong premating isolation between the pure species, female hybrids were attractive to males of either species, and male hybrids were attracted to any female. Similar results on hybrid sexual attractiveness have been obtained in a number of other butterfly species (e.g. *Heliconius*: McMillan *et al.* 1997, Naisbit *et al.* 2001).

It was not possible to continue the crosses, but some clear facts emerge. Firstly, F_2 hybrid breakdown is not absolute in interspecific crosses. Secondly, it is not limited to interspecific crosses; it may take place between subspecies, as is known in other species (e.g. Oliver 1972, 1978, Jiggins *et al.* 2001). The latter is particularly paradoxical, since, within both species, broad, clinal, unimodal hybrid zones connect 'incompatible' populations. Careful field work could well disclose interesting features in these contacts. Hybrid inviability is therefore probably not a useful species criterion on its own in crosses between geographically distant taxa. The ease of playing ping-pong with the two species once initial barriers have been ruptured shows that there is no absolute threshold of postzygotic incompatibility at the species level.

Frequent hybridization and introgression in sympatric Papilionidae: *Papilio machaon* and *P. hospiton*; *Parnassius apollo* and *P. phoebus*

PAPILIO MACHAON AND *P. HOSPITON*

Hybridization is widespread in *Papilio* species, especially in North America (Sperling 1990). Hybrids between the Eurasian *Papilio machaon* and the endemic *P. hospiton* of Corsica and Sardinia have been known for a long time (e.g. Verity 1913). Although their habitats and distribution in Corsica are very different, there is a frequent overlap, and hybridization occurs regularly. Crosses revealed two especially important postzygotic barriers (Clarke & Sheppard 1953, 1955, 1956, Clarke & Larsen 1986). (1) An almost total inviability of $F_1 \times F_1$ hybrid crosses, originally mistaken for F_1 sterility. However, non-hatching ova were not 'sterile'; instead embryos show arrested development at various stages between early segmented embryos and fully-developed larvae unable to break out of their egg shell. (2) Strong Haldane's rule F_1 hybrid effects. In *hospiton* male × *machaon* female crosses reared in Britain, female hybrid pupae became 'perpetual nymphs', that is pupae which are unable to resume development. However, in other *Papilio* interspecific hybrids with extended diapause, ecdysone and insulin injections can trigger development (Clarke *et al.* 1970, Arpagaus 1987). Descimon & Michel (in Aubert *et al.* 1997) showed that insulin could also trigger development in *machaon* × *hospiton* hybrids.

Both reciprocal F_1 crosses and various backcrosses proved possible. The experiments were carried out in the Paris region, in an oceanic climate, and in Marseilles, on the Mediterranean, but under long photoperiod summer in both cases (Aubert *et al.* 1997). In the case of *hospiton* male × *machaon* female crosses, results depended on rearing conditions. In Paris, growth and developmental time of males was normal, but the female pupae, which were markedly bigger than those of either parental species, became perpetual nymphs, as found by Clarke & Sheppard (1953). In Marseilles, females did not enter diapause and gave large, viable females. The other possible F_1 (*hospiton* female × *machaon* male) again gave healthy hybrid males, but females were small, with accelerated development and no diapause, in both climates. $F_1 \times F_1$ crosses gave almost complete inviability at various stages of early development, as before. On the other hand, backcrosses were all viable. F_1 hybrid females, in particular, appeared not to be sterile, whether they had *hospiton* or *machaon* as mothers.

The results suggest that introgression is possible. Allozyme and restriction fragment length polymorphism (RFLP) analysis of mtDNA markers show strong differentiation between the two species, with diagnostic alleles at some loci and a rather high Nei's *D* and mtDNA sequence divergence (Aubert *et al.* 1997, Cianchi *et al.* 2003). Putative hybrids found in different localities in Corsica and Sardinia were most probably F_1s, and from both reciprocal crosses. No individuals were found with introgressed mtDNA RFLP types in a large sample, suggesting a lack of mitochondrial introgression. However, the same was not true for nuclear loci. Alleles from *hospiton* were found in Corsican *machaon*, but were always absent in continental *machaon* (Aubert *et al.* 1997, Cianchi *et al.* 2003). The frequency of hybrids was lower in the Italian than the French data set (approx. 1% vs. 5%), but this is probably because HD collected especially avidly in areas of cohabitation, whereas many samples obtained by the Italians contained only one species.

Classically, *hospiton* is considered single-brooded, while *machaon* is multi-brooded. However, broods reared from wild Corsican *hospiton* females give a proportion (5–100%) of non-diapausing pupae (Aubert *et al.* 1996a). Diapause control in *P. hospiton* (and in *P. machaon*) is highly heritable but not simple; temperature and photoperiod act in combination, with threshold effects which interact strongly with genetic factors. Multi-brooded individuals are particularly common where *hospiton* feeds on *Peucedanum paniculatum*, a perennial evergreen umbellifer endemic to northern Corsica; this plant is suitable throughout the warm season. Observations in July and August confirm the existence of the second brood (Aubert *et al.* 1996a, Guyot 2002, Manil & Diringer 2003). In most regions of Corsica and throughout Sardinia, the main foodplant, *Ferula communis*, withers down as early as May onwards. Even here, late larvae can be found when roadside mowing during late summer renders resprouting *Ferula* available (Descimon, pers. obs.).

Aubert *et al.* (1997) suggest that multivoltinism in *P. hospiton* may result from introgression from *P. machaon*. This hypothesis was criticized by Cianchi *et al.* (2003) because of doubt in the existence of the second brood of *P. hospiton* (this argument is not tenable, as we have seen). Of more weight is the difficulty of distinguishing ancestral from introgressed polymorphisms. Nonetheless, Cianchi *et al.* (2003) found up to 43% *hospiton* allozymes in *machaon* on the islands, though never present on the mainland, and they argued that this was due to introgression. Conversely, they found only a scattering of *machaon* alleles in *hospiton*. They argued that this introgression was mostly ancient and that reinforcement of interspecific barriers took place early during the secondary contact. This conforms to the commonsense prediction that what we observe today is an equilibrium between gene flow and selection against introgression (Descimon *et al.* 1989).

PARNASSIUS APOLLO AND *P. PHOEBUS*

Parnassius apollo is a montane butterfly, widespread from Altai in central Asia to the Sierra Nevada in southern Spain. *Parnassius phoebus* has a more restricted, higher-elevation distribution; in Europe, it occurs and can hybridize with the *P. apollo* only in the Alps (Plate 19b). The species always occur in close proximity (dry, sunny slopes for *P. apollo* and banks of torrents and rills for *P. phoebus*), but this does not ensure hybridization. Not only are their preferred flight environments different, but *P. phoebus* also flies earlier in the year. Therefore, it is only in localities where the two kinds of habitats are closely interspersed and phenology is perturbed that hybridization takes place, often at rather high frequency (Descimon *et al.* 1989). In some localities, hybrids are observed almost yearly; in others, they occur only following a snowy winter, when avalanches accumulate in the bottom of thalwegs. Thus, rather 'soft' pre-mating barriers, such as habitat and phenology differences, prevent hybridization. In captivity, mating between male *apollo* and female *phoebus* is often observed, and hand-pairing easy. The reverse cross is more difficult, due to the small size of male *phoebus*. F_1 hybrids display typical vigour and females are not perturbed in diapause (which takes place in the first larval instar, inside the egg shell). Field observations on wild hybrids show a strikingly perturbed behaviour: males fly restlessly, constantly roaming between the types of habitat preferred by both parent species. In captivity, male hybrids backcross freely with females of both species and are highly fertile, but female hybrids are inevitably sterile, producing numerous small ova that never hatch.

Morphometric analyses of natural populations strongly suggested backcrossing as well as F_1 hybrids in the field (Descimon *et al.* 1989). Using four diagnostic allozymes and several other loci with different allele frequencies in the two species, F_1 hybrids and backcrosses were detected (Descimon & Geiger 1988). One individual with the pure *apollo* wing pattern was heterozygous at one of the diagnostic loci, suggesting that backcrossing continues beyond the F_2. Mitochondrial DNA analysis showed that hybridization took place in both reciprocal directions but also that backcrossing could involve hybrid females (Deschamps-Cottin *et al.* 2000). While this contradicts findings from some captive broods (Descimon *et al.* 1989), it conforms to others (Eisner 1966). Once again, introgression in nature seems possible and is demonstrated by the field results.

COMPARISONS BETWEEN THE TWO HYBRIDIZING PAIRS OF PAPILIONIDAE

It seems clear that most would regard the four swallowtails treated here as four distinct, if somewhat bad species. They are readily distinguishable on the basis of morphology, allozymes and mtDNA. Allozyme and mitochondrial divergences suggest an age of around 6 Myr for the *Papilio machaon–P. hospiton* pair (Aubert *et al.* 1999), and a similar age is probable for *Parnassius apollo* and *P. phoebus*. Regular hybridization is therefore not necessarily a sign of incomplete speciation, but rather of the inability of the taxa to erect complete pre-mating barriers.

In conclusion, species can remain stable in spite of frequent hybridization and introgression. While there has been significant progress in understanding this introgression, we still have little overall knowledge of the genomic distribution of introgressed and non-introgressed loci.

Polyommatus (Lysandra) coridon, L. hispana and *L. albicans*: frequent hybridization everywhere, strong gene flow and yet species remain distinguishable!

For a long time, the chalkhill blue was considered in Europe to be a single species, *L. coridon*. However, in *Polyommatus sensu lato*, species rarely show consistent differences in genitalia or wing pattern (Plate 19a). Because of this, complexity in the *coridon* group was recognized initially due to voltinism. In 1916, Verity observed three emergences of *Lysandra* in the hills around Florence, Italy and showed that this was due to the existence of two separate species: one single-brooded, *coridon sensu stricto*, one double-brooded, *hispana* H.-S. Later on, he recognized *L. caelestissima*, univoltine with a distinctive sky-blue colour, from Montes Universales, central Spain. In Spain, the situation is especially confusing: there are single- and double-brooded forms, and bimodal hybrid zones where they overlap. At one time, clear blue hybrids between *L. caelestissima* and *L. albicans* from Montes Universales were also considered a distinct species, *caerulescens*. For a while the number of species recognized varied from one to four; eventually three were recognized on the basis of chromosome number and voltinism (de Lesse 1960a, 1969). These are:

(1) *Lysandra coridon*: widespread, univoltine, with $n = 88–90$, with an isolate in central Spain, *caelestissima*, considered a subspecies with $n = 87$.
(2) *Lysandra albicans*, univoltine, southwestern Spain, $n = 82$.
(3) *Lysandra hispana*, central France and Italy to Northern Spain, bivoltine, $n = 84$.

De Lesse (1969) described ssp. *lucentina* (correctly: *semperi* Agenjo 1968) from the Alicante region, which he referred to *hispana* on the basis of chromosome number ($n = 84$); later it turned out to be univoltine like *albicans*. He also showed that *L. italaglauca*, described as a species from central Italy, was actually a rather abundant hybrid between *L. coridon* ($n = 88$) and *L. bellargus* ($n = 45$). This form, of intermediate colour between the greyish of *L. coridon* and the dazzling blue of *L. bellargus*, was identical to *L.* × *polonus* (Zeller 1845), formerly mistaken as a good species from Poland and later recognized as a hybrid (Tutt 1910). These hybrids occur wherever the parent species fly together, although their frequency varies widely. *Lysandra coridon* is univoltine and flies around August, while *L. bellargus* is bivoltine and flies in May and September; the hybrid flies in late June. The meiosis of these hybrids displays incoherent equatorial plates, strongly suggesting sterility (de Lesse 1960a). Ironically, a blue species, *L. syriaca*, from the Middle East was for a while mistaken for *polonus* (Lederer 1858). Tutt (1914), who had earlier deduced that *polonus* was a hybrid, also correctly interpreted *L. syriaca* as a 'good' species. By analogy, de Lesse interpreted *L. caerulescens* as a hybrid, but, in this case, karyotypes are similar and meiosis appears normal. Laboratory hybrids between *L. coridon* and *L. hispana* obtained by Beuret (1957) proved fertile and viable until the F_3 generation. Interestingly, individuals from the last generation had the most chromosomes, as in *Antheraea* moths (Nagaraju & Jolly 1986). Another 'blue' hybrid mistaken for a species, famous for the author who described it, '*Lysandra*' *cormion* (Nabokov 1941), turned out to be a *Lysandra coridon* × *Meleageria meleager* hybrid (Smelhaus 1947, 1948, Schurian 1991, 1997). Again, hybridization occurs regularly in some regions (Moulinet, Alpes Maritimes, France; Bavaria, Germany).

De Bast (1985) followed up de Lesse's work using morphometric analysis on imaginal morphology and wing pattern. He recognized five species, *L. coridon*, *L. caelestissima*, *L. albicans*, *L. hispana* and *L. semperi*. The latter could be referred either to *hispana* via karyotype and wing pattern or to *albicans* via voltinism. In 1989, Schurian, after breeding experiments, crosses and morphological studies of all instars from egg to imago, recognized only three species, *coridon*, *albicans* and *hispana* (*semperi* was included within *hispana*).

Based on a restricted sample of 15 populations, Mensi *et al.* (1988) separated *coridon* and *caelestissima* as species because of a diagnostic allozyme (*Pk-2–105*), absent in *caelestissima*. Lelièvre (1992) systematically sampled 75 populations, collected by himself and HD, in order to cover all known systematic units and to test for hybrid zones in France and Spain. Allozyme analysis showed that two main entities could be readily distinguished: *coridon* + *caelestissima*, and *hispana* + *albican*s + *semperi*, with Nei's $D \approx 0.05$ between the two groups. In contrast, *L. bellargus* was separated from the *coridon* group by a $D \approx 0.30$. No diagnostic alleles were found between *coridon* and *caelestissima*, contradicting Mensi *et al.* (1988). Therefore, there is little reason to consider them as separate species. The chief argument for separation is the colour of male imagines, but, in northern Spain, populations are often of intermediate colour (ssp. *manleyi* and *asturiensis*). A sex-limited morph, the blue '*syngrapha*' female, shared by *coridon* and *caelestissima* (Descimon 1989) also suggests conspecificity. Disjunct distributions of the two taxa prevent use of the cohabitation criterion. A conservative solution is thus to merge all the populations into a single species with some strong subspecies.

The tale of *L. coridon* in Tyrrhenian Islands is almost incredible. Its lime-loving foodplant, *Hippocrepis comosa*, is very scarce on the mainly acidic soil of these islands. The description in 1977 of ssp. *nufrellensis* from the remote granitic Corsican Muvrella massif by Schurian attracted scepticism, but was confirmed in 2006 by Schurian *et al.* – Muvrella granite is hyperalkaline and supports *H. comosa*! *L. coridon*, described as *gennargenti*, was also found in Sardinia on more easily accessed calcareous patches (Leigheb 1987). Both populations are well characterized by adult wing pattern (the males are vivid blue and females are always blue) and by preimaginal stages. Marchi *et al.* (1996), using allozyme analysis, left the form as a subspecies of *coridon*. However, Jutzeler *et al.* (2003a, b) did not lose an opportunity to raise yet another known form to species rank, based only on preimaginal morphology.

In the '*hispana–semperi–albicans*' complex, things are much more complicated. Populations assigned to one of these putative taxa by 'classical' criteria (namely, wing pattern, distribution and voltinism) are not distinguishable via allozymes. This is especially true for '*albicans*' and '*semperi*', which broadly overlap in their allozyme polymorphisms.

Hybrid zones between the taxa give rise to additional complexity. A hybrid zone exists between *caelestissima* and *albicans* in Montes Universales (central Spain); both are single-brooded and fly at the same time of year. The former flies at rather high elevation (1200–1800 m), the latter in lower zones (800–1400 m). They overlap at intermediate altitudes, where putative male hybrids ('*caerulescens*') can easily be detected by wing colour. We have studied three samples, each containing ~30 individuals: the first from a pure *caelestissima* locality (Paso del Portillo); the second from an *albicans* locality (Carpio del Tajo); and a third area of cohabitation, where hybrid *caerulescens* reach a frequency of 10% or more (Ciudad Encantada). Allozyme genotypes were concordant with colour pattern in 77% of the cohabiting sample. Discordant individuals were all '*caerulescens*', that is, presumably hybrids, and their allozyme genotypes were intermediate (Lelièvre 1992). The hybrid zone thus appears more or less bimodal, even though hybrids were rather abundant.

Two other hybrid zones were studied in northern Spain (at Ansó and Atarés in the Jaca region), where single-brooded *L. coridon manleyi* overlaps with double-brooded *L. hispana*. The former species again flies at a higher elevation, but the two overlap at intermediate altitudes. 'Pure' reference populations were again studied nearby: Aranqüite and Embalse de Oliana, respectively. In the hybrid zone at Ansó, the variously coloured butterflies were hard to separate genetically. Individuals were either genetically similar to those from one or other pure sample, or intermediates. In the second hybrid zone, at Atarés, two visually different categories of individuals were found, some with the obvious clear blue *coridon* phenotype, the others greyish-white and similar to *hispana*. Intermediate specimens were scarce and none was analysed genetically. Paradoxically, all genotypes from the cohabitation zone, including those classified as *hispana* by wing pattern, corresponded to *coridon* from Aranqüite, rather than to *hispana* from Oliana, so introgression is suspected (Lelièvre 1992).

More recently, bivoltine *Lysandra* populations flying in southern Slovakia were separated out as a species, *Polyommatus slovacus* (Vitaz *et al.* 1997), on the basis of subtle adult morphological differences (the bluish dorsal hue of male wing pattern and slight differentiation of male and female genitalia). A cohabitation criterion was used, since it apparently flies with univoltine *L. coridon* in some localities, although there is no mention of hybrids. There is no known genetic difference between *L. slovacus* and neighbouring populations of *L. coridon* (Schmitt *et al.* 2005). Voltinism remains the chief character.

In conclusion, there is one rather clear, homogeneous species, *L. coridon*, with strongly differentiated subspecies in Spain (*caelestissima*) and the Tyrrhenian Islands (*nufrellensis*); chromosome characters and phenology as well as allozyme data support the unity of this taxon. The geographically variable male wing colour pattern conforms to this diagnosis, since populations from northern Spain are intermediate. In contrast, the same criteria do not provide coherent evidence for splitting the *hispana* complex into several units. The forms *semperi* and *hispana* share the same karyotype ($n = 84$), but the former is univoltine like *albicans*, which, however, has a different chromosome number ($n = 82$). Allozymes have not yet proved very useful. HD has doggedly sought further contact zones between the three taxa of the *hispana* complex, but in vain. Lelièvre's (1992) work was extremely useful, but his premature death prevented a more complete analysis.

The *Erebia tyndarus* group: parapatry, hybrid zones and Gause's principle

This group (Plate 20c) illustrates the use of successively more sophisticated taxonomic criteria, and the difficulties of applying various species concepts; we therefore employ a historical approach. The *tyndarus* group is characterized by cryptic grey hind wing undersides, which provide good camouflage in rocky grasslands. Their distribution stretches from western North America, across the Pacific to Eurasia, and finally to the Asturias in Spain. Until the twentieth century, all were considered to belong to a single variable species. In 1898, Chapman piloted the use of male genitalia in *Erebia* and recognized *E. callias* Edwards from North America, and a submontane form from Asia Minor, *E. ottomana* H.-S., as separate species. In 1908, Reverdin studied wing pattern in Western European taxa, and showed that the Alpine forms could be arrayed in two groups, *E. tyndarus* Esper and *E. cassioides* Reiner & Hohenwarth. The latter can also be recognized in the Pyrénées, Apennines, Balkans and Carpathians. He further noted that the southernmost form, *hispania* Butler from the Sierra Nevada, could be grouped with others from the Pyrénées, *goya* Frühstörfer and *rondoui* Oberthür, without elevating them to species rank.

Warren (1936) recognized four species based on male genitalia: *tyndarus*, *cassioides*, *dromulus* Staudinger (from the mountains of Asia Minor) and *callias*, from North America, Central Asia, Elburz and the Caucasus. In 1949, he pointed out that *cassioides* and *rondoui* (previously included with *tyndarus*) overlapped in the Pyrénées and considered this cohabitation evidence for separate species. In 1954, he extended this to *tyndarus sensu stricto* on the grounds of cohabition with *cassioides* in the Bernese Alps.

There is a striking feature in the *tyndarus* group: distributions of the taxa are typically parapatric and in a given region, there is only one form. Distributions overlap only in very narrow contact zones. Sometimes, hybrids are found in various proportions (see below); in other cases, hybridization is absent. Mutual exclusion can be attributed to Gause's (1934) principle: 'one species per ecological niche'. For the BSC, the *tyndarus* group was somewhat distressing: morphological criteria are weak, and ecological differences minimal, as shown by mutual geographical exclusion. Narrow cohabitation with little or no admixture therefore became the main distinguishing criterion within this group.

Warren never went beyond genitalic characters, but de Lesse and Lorković initiated a synthetic approach using karyotype, morphometrics of genitalia, wing-pattern variation, laboratory crosses, and detailed field studies on distribution and hybrid zones. There was great variation in chromosome number: *hispania*, with $n = 24$, stood out from *cassioides* and *tyndarus*, with $n = 10$ throughout their ranges (Lorković 1949, 1953, de Lesse 1953). Later, two cryptic species were discovered: *calcaria* Lrk. ($n = 8$), from the Julian Alps, and *nivalis* Lrk. & de Lesse ($n = 11$), limited to upper elevations of the Eastern Alps, where it flies above *cassioides* or *tyndarus* (Lorković 1949, Lorković & de Lesse 1954b). In addition, de Lesse (1955a, c) showed that *E. callias* from North America and *E. iranica* and *E. ottomana* from the Middle East displayed markedly different karyotypes ($n = 15$, 51, and 40, respectively). De Lesse (1960a) performed morphometric analyses of genitalia. He reinstated wing pattern as a valuable tool if concordant with other characters. In particular, he noticed that the dark hind-wing eyespots could be shifted distally in their fulvous surrounds, rather than being centred, enabling one to group the southernmost taxa, *hispania* and *iranica*, also characterized by high chromosome numbers ($n = 24$–25 and 51–52). Recent studies have shown that satyrine eyespot variation often results from important developmental genetic shifts (Brakefield 2001). Locally adaptive camouflage wing patterns (see above), such as hind-wing underside colour, provided less useful criteria.

Lorković (1954) carried out crosses between several taxa (*calcaria* × *cassioides*, *calcaria* × *hispania* and *cassioides* × *ottomana*). All showed genetic and behavioural incompatibility: assortative mating, together with sterility of primary crosses and of F_1 hybrids (Lorković & de Lesse 1954a).

However, the taxa used were not the most significant: *ottomana* is notoriously distant from the other members of the group (see below); *calcaria* and *hispania* differ in karyotype ($n = 8$ and 24 respectively) and their ranges are very distant. The most useful test is *calcaria* × *cassioides*: they have identical karyotypes ($n = 10$) and adjacent distributions, but clear incompatibilities were still found.

It was thus important to investigate contact zones and distribution in nature. A complex pattern of allopatric distribution of *hispania* and *cassioides* was found in the Pyrénées (de Lesse 1953, Descimon 1957), with very narrow zones of cohabitation. Only a single putative hybrid was captured by Descimon (de Lesse 1960a) among several hundred individuals in many zones of overlap. In the central Alps, *tyndarus* occurs as an outpost inserted between two disjunct populations of putative '*cassioides*'. In the absence of differences in chromosomes, genitalia and wing pattern provided the only useful criteria. Westwards, in Val Ferret, southwest Switzerland and in adjacent Italy, above Courmayeur, populations of *tyndarus* and '*cassioides*' are separated by narrow unoccupied regions (de Lesse 1952). Near Grindelwald, in the Bernese Oberland, a cohabitation site with phenotypically intermediate individuals was found. At the eastern end of the *cassioides–tyndarus* contact zone, in Niedertahl, Austria, a cohabitation site was found, but hybrids were not found, even though enhanced variability in genitalia suggested introgression (Lorković & de Lesse 1955).

Erebia nivalis Lrk. & de L., originally considered a smaller high-elevation form of *cassioides* (Lorković & de Lesse 1954b), was raised to species rank after discovery of its peculiar karyotype ($n = 11$). Cohabitation is often observed at the altitudinal boundary between the two, although hybrids are never found. Competitive exclusion is especially convincing: at Hohe Tauern, a different species occurs on each of two isolated massifs (*cassioides* on Weisseck and *nivalis* on Hochgolling); in both cases the entire span of alpine and subalpine zones (1800–2600 m) is occupied, suggesting competitive release (Lorković 1958). Similarly, in eastern parts of their distribution, *cassioides* and especially *tyndarus* reach higher elevations in the absence of *nivalis*. The distribution of *nivalis* is broadly fragmented into two parts: in the Austrian Alps and in a more restricted area in the Bernese Oberland. The gap between the two areas occupied by *nivalis* has been colonized by *tyndarus*. In the Grindelwald area, where all three taxa cohabit, *tyndarus* looks like the more aggressive competitor which has eliminated *nivalis* even from high-elevation habitats.

A rather clear picture emerges from these studies (Guillaumin & Descimon 1976): in Europe, the *tyndarus* group includes several well-defined species: *ottomana, hispania, calcaria* and *nivalis*. The *tyndarus–cassioides* pair is more puzzling. By now, a disjunct assemblage of seemingly subspecific forms were recognized as *cassioides*, including populations from the Asturias, the Pyrénées, Auvergne in French Massif Central, Western and Southern Alps, Eastern Alps, the Apennines and some Balkan massifs. The populations referable to *tyndarus* occurred in a continuous distribution inserted like a wedge between *cassioides* populations in the Central Alps. Lorković (1953) proposed that these taxa were examples of an intermediate category, 'semispecies' (Lorković 1953, Lorković & Kiriakoff 1958). However, in practice, *cassioides* and *tyndarus* were considered separate species by most lepidopterists (e.g. de Lesse 1960b).

In 1981, Warren published a supplement to his monograph of the genus *Erebia*. Arguing that chromosomes had little systematic value, he relied mainly on male genitalia and arranged the taxa in a somewhat confusing way. This was accentuated because he considered *cassioides* a *nomen nudum*, in spite of the lectotypification of the figure in Reiner & Hohenwarth by de Lesse (1955a) – he considered the figure was inaccurate. He recognized the following European species:

(1) *tyndarus* – Central Alps.
(2) *nivalis* – Austrian Alps and Bernese Oberland.
(3) *aquitania* Frhst. (= *cassioides pro parte*) – Southern Alps, Dolomites, Karawanken, Montenegro, Etruscan Apennines, Mont Blanc range and Pyrénées (part).
(4) *neleus* Frr. (= *cassioides pro parte*) – Transylvanian Alps, Austria, Rhodope, Macedonia, Central Alps, Pyrénées (part), Roman Apennines, Abruzzi, Auvergne.
(5) *calcarius* – Julian Alps.
(6) *hispania* – Sierra Nevada and Pyrénées.
(7) *ottomana* – considered very distinct from the other members of the group.

The species designated by Warren in the former *cassioides* group lacked zoogeographical coherence compared with those recognized by de Lesse & Lorković. The only serious (partial) support for Warren's theses was the suggestion that populations of *cassioides sensu lato* east of the *tyndarus* wedge could be called *neleus*, and the western ones *aquitania* (von Mentzer 1960). This prophetic suggestion, making zoogeographical sense, was largely overlooked at the time.

A much firmer position was adopted by Niculescu (1985): an extreme 'lumper', he used only morphological criteria to unite all of the group in a single polytypic species, *tyndarus*. Much earlier, de Lesse (1960a: 57), had warned about the exclusive use of morphology as criteria to delimit species, especially if already known to be labile and if the classification required illogical zoogeographical distributions. However, Gibeaux (1984) claimed he had discovered *E. calcaria* and *E. tyndarus* closely adjacent to *cassioides* in the Col Izoard region of the French Alps, on the base of wing pattern and genitalic morphology, without reference to karyotype, cohabitation and molecular criteria. Lorković (pers. comm. to HD) keenly argued that the genitalic characters used by Gibeaux could be explained by individual variation. Wing-pattern differences were confined to the strongly selected, taxonomically useless hindwing undersides.

Ten years later, a far more informative study, based on 17 allozyme loci, largely confirmed the common ground of previous authors: *ottomana*, the *hispania* complex and *nivalis* were very distinct from other members of the group, with Nei's $D > 0.20$ (Lattes *et al.* 1994). The single available sample of *tyndarus* differed by $D = 0.14$ from the cluster, while '*cassioides*' itself consisted of clearly differentiated 'western' and 'eastern' *cassioides* groups. Lattes *et al.* attempted to outflank Warren's rejection of the name *cassioides* by designating a neotype; an actual museum specimen from the Austrian Alps – *cassioides sensu stricto* therefore now refers specifically to the eastern taxon. Actually, the older valid name for western '*cassioides*' was *arvernensis* Oberthür (type locality: northern French Massif Central), and we use it instead of *neleus* below. The rather large genetic distance between *hispania sensu stricto* from Sierra Nevada and *rondoui* and *goya* from the Pyrénées (Nei's $D = 0.16$), added to slight differences in chromosome number ($n = 25$ vs. 24, respectively), led the authors to consider them different species. However, they did not do the same with two *ottomana* samples from the Italian Alps and southern French Massif Central, even though they were distant by a Nei's D of 0.18.

Most recently, a study using allozymes and sequence data from two mtDNA genes was carried out on a limited number of populations (Martin *et al.* 2002); eastern '*cassioides*', in particular, was lacking. There were large genetic distances between *ottomana* and *hispania sensu lato*, and their monophyly was confirmed; *tyndarus* (three populations) also proved monophyletic, while *nivalis* formed a strongly supported group together with *calcaria*; divergence at the mtDNA genes averaged 0.34%. The allozyme data showed a similar pattern to that found by Lattes *et al.* (1984): *nivalis* was located at the end of a long branch. In contrast to *tyndarus*, *arvernensis* did not group as a single cluster and appeared paraphyletic. The basal and terminal branches of these trees were well resolved, but the intermediate branches, which should define the phylogenetic relationships between *tyndarus*, *arvernensis*, *nivalis* and *calcaria*, remained unclear. The lack of eastern *cassioides sensu stricto* prevented accurate phylogenetic estimation, since we still do not know if this taxon clusters with *arvernensis*, *tyndarus*, or *nivalis* and *calcaria*.

A final and rather ludicrous episode of this tale occurred in the butterfly distribution atlases for France (Delmas *et al.* 1999) and Europe (Kudrna 2002). The former used the correct name *arvernensis* for 'western *cassioides*'. The resultant geographical distributions were correctly documented by Kudrna, but this author also reported older literature records from France (as well as from Spain, parts of Switzerland and Italy) as '*cassioides*'. Hence an extensive but entirely fictitious pseudo-sympatry of the two taxa was reported in the French Alps and Pyrénées, and even in the northern Massif Central.

Erebia serotina Descimon & de Lesse, 1953: a hybrid mistaken for a species

In September 1953, the 19-year-old HD captured two individuals of an unknown *Erebia* at 1000 m elevation in the Pyrenean valley of Cauterets and showed them to H. de Lesse. After careful examination, they concluded that the butterflies belonged to an unknown, late-flying species they named *E. serotina* (Descimon & de Lesse 1953) – a surprising finding in the mid twentieth century. Further individuals were captured regularly in the same region over a period of 10 years, always late in the season and at the same elevation (Descimon 1963) (Plate 20a). Chromosome study (Descimon & de Lesse 1954) disclosed a number of $n = 18$.

However, the absence of females in a sample of 18 individuals was intriguing; Bourgogne (1963) suggested that *E. serotina* was a hybrid between *E. epiphron* and *E. pronoe*, both also present in the region and having chromosome numbers of 17 and 19, respectively. This possibility had been rejected by Descimon & de Lesse, since the two species live at a higher elevation than *serotina* (over 1400 m and above the treeline). Moreover, de Lesse and later Lorković (pers. comm. to HD), who examined the histological preparations of *serotina* testes, considered chromosome pairing during meiosis to be normal. The debate was echoed by Riley (1975) and Perceval (1977), with no additional data. Higgins & Riley (1970) included *E. serotina* in their field guide, although the species was not mentioned in later editions or other guides.

A few other specimens were captured in the same valley (Lalanne-Cassou 1972, 1989) and 15 km to the west (Louis-Augustin 1985) and also in the Spanish Pyrénées, always late and at low elevation (Lantero & Jordana 1981). Warren (1981) was also inclined to the hypothesis of a hybrid, which he considered to be between *epiphron* and *manto*, another Pyrenean species, on the basis of morphology and against the chromosomal evidence – *manto* has $n = 29$, which should yield $n = 23$ for the hybrid. At this juncture, both 'hybrid' and 'good species' hypotheses seemed unlikely.

Forty years later, the retired HD again went in pursuit of *serotina* and found several individuals in September 2000 and 2002 close to Bagnères de Luchon, 60 km east of Cauterets (Descimon 2004). An analysed individual was heterozygous at all diagnostic allozyme loci between *epiphron* and *pronoe*, while mtDNA showed that *epiphron* was the mother (E. Meglécz *et al.* unpublished). Therefore, *serotina* is indeed a hybrid between *epiphron* and *pronoe*. Moreover, after a series of hand-pairing crosses, three hybrids similar to wild *serotina* were obtained by Chovet (1998). Bourgogne's hypothesis was therefore proved correct and the mystery of *Erebia serotina* solved; the absence of females may be due to arrested growth, while males undergo accelerated development and hatch before the cold season (see the *Papilio* case above). Now, the riddle has moved on towards other questions: why does *serotina* fly at altitudes where its parents do not? Why does it occur regularly in the Pyrénées, but not in other regions of parental contact?

Hybrids are scarce in *Erebia*: apart from the previously mentioned *arvernensis* × *hispania* hybrid, only two other cases have been recorded. The first, *intermedia* Schwnshs, is found in the Grisons, Switzerland; initially mistaken for a variety of *E. epiphron*, it was later shown to be a *flavofasciata* × *epiphron* hybrid (Warren 1981). The second has been collected only once, from the Carpathians, and was recognized immediately as a *pronoe* × *medusa* hybrid (Popescu-Gorj 1974). Taken in late September, like *serotina*, it was similar to it also in its genitalia. In all three cases, at least one of the parents of *serotina*, *E. epiphron* or *pronoe*, is involved.

Other cases of 'bad' species in European butterflies

Palaearctic butterflies demonstrate many other cases of uncertain or 'fuzzy' species (Tolman & Lewington 1997) (Table 16.1B). These cases suggest some general patterns of 'bad' species relations, often involving hybrid zones. Some such zones present ecological frontiers, in particular at boundaries between lowland and montane taxa: *Pieris napi* and *bryoniae*, *Euchloe crameri* and *simplonia*, *Lycaena tityrus* and *subalpina*, *Melitaea parthenoides* and *varia*, *Coenonympha arcania*, *gardetta* and *darwiniana*, *Pyrgus cirsii* and *carlinae*. *Coenonympha darwiniana* may actually be a stabilized hybrid between *arcania* and *gardetta*, since it is found at intermediate elevations between the areas where *arcania* and *gardetta* occur (Holloway 1980, Porter *et al.* 1995, Wiemers 1998). In most cases, the limit coincides with the elevation where two broods per year become impossible because of low mean temperature; a similar phenomenon in latitude is found in most areas where *Aricia agestis* meets its congener *artaxerxes*. Very often, there is a gap where neither form is regularly present, perhaps because in this area, a second brood can be triggered by photoperiod, but does not complete its growth before autumn, and fails. Here, a discrete biological response cannot easily track a continuous environmental change. Another striking feature is that differentiation between clearly distinct taxa is often observed in the Alps, while in the Pyrénées similar distribution gaps are observed, but with much weaker genetic differentiation between single- and double-brooded populations (e.g. *L. tityrus* and *M. parthenoides*). The case of *Maculinea alcon* and *M. rebeli* is so complex and the ecology of both taxa has given rise to so many papers that it deserves separate treatment. The case of these blues is the closest in butterflies to 'ecological races'. No differences were found at mtDNA or nuclear EF1-α gene sequences (Als *et al.* 2004). However, we know too little about gene exchange between the populations to locate them with precision on the bad species–good species spectrum (Wynhoff 1998, Als *et al.* 2004).

Other repeated patterns in contact zones suggest 'suture zones' (Remington 1968) caused by secondary contact of whole faunas from different Pleistocene or earlier refuges, especially the Iberian ('Atlanto-Mediterranean'), and Italian + Balkans refuges ('Ponto-Mediterranean': de Lattin 1957). *Iphiclides podalirius* and *feisthameli*, *Pontia edusa* and *daplidice*, *Colias hyale* and *alfacariensis*, *Lycaena alciphron* and *gordius*, *Melitaea athalia* and *celadussa*, and *Melanargia galathea* and *lachesis* appear to belong to this category. Desert species such as *Papilio saharae* and *Melitaea deserticola* meet with temperate counterparts in northern Africa, while montane species also provide examples of differentiation in various refuges followed by subsequent contact. A general feature of these contacts is Gausean exclusion and therefore parapatry; the cases of *Erebia pandrose* and *sthennyo*, *E. euryale* forms, *mnestra* and *aethiopellus* are comparable with the *tyndarus* group in this respect. Finally, Corsican and Sardinian endemics are somewhat different; they might be expected to provide parallels with *P. machaon* and *hospiton*, but they lack genetic differentiation or pre- and post-mating incompatibility; consequently, they are not able to cohabit.

GENERAL DISCUSSION

The examples studied here can serve as a testbed for theories and concepts of species and speciation, and of their use in answering questions such as: are there one, two, or more 'good' species involved, or is this an example of speciation in progress? Can we use the results to suggest a simple and unequivocal, or at least useful nomenclature? Is there a general procedure, using the tools and concepts already mentioned, to allow us to reach this goal?

The simplest case is *Erebia serotina*. Originally ranked as a species, it ended up as a mere hybrid: 1→0. Here the difficulty was technical: it was finally through the use of molecular markers that the parent species and the sexes involved in the cross were recognized. In the case of *Lysandra polonus* and *L. italaglauca*, the tools were cytological; in these cases, the sex of the parents involved remains unknown, although mtDNA analysis could easily solve the question. Among many other known hybrids (Table 16.1), the majority have been identified only via wing pattern. There is an opposite case, where a species, *Lysandra syriaca*, was recognized after being initially confused with the hybrid *polonus*: 0→1. Hybridization does not occur in all zones of cohabitation with the same frequency, as seen in all the cases studied here. The behaviour of hybrids can be not only different from either parent, but also not intermediate; this is especially striking with *serotina*, but is also observed with *Parnassius apollo* × *phoebus* hybrids (Descimon *et al.* 1989).

With *L. sinapis* and *reali*, we have an opposite, but equally clear case: 1→2. The data provide an unambiguous result under all species concepts: there are clear morphological differences; gene pools are completely isolated (to satisfy BSC adepts); the ecological niches are different and the two species form mutually monophyletic assemblages and thus raise no problem for phylogeneticists.

Things become more complex with *Zerynthia*. Few doubt that *Z. rumina* and *polyxena* are 'good' species. Again, there are obvious morphological differences, and there is a rather strong separation of gene pools – hybrids are scarce enough to satisfy BSC groupies, in spite of broad sympatry and character displacement in ecological preferences. Phylogeneticists will be happy that each species constitutes a monophyletic assemblage. However, serious genomic incompatibilities were observed between distant populations within each of these species, especially within *rumina*. In fact, the level of incompatibility between the species was not markedly greater than within each. So does *Zerynthia* contain one, two, three, four or even more species? These findings occurred only as a result of crosses between forms which do not co-occur naturally; they are artefacts. Similar incompatibility effects have also recently been observed within the well-known tropical species *Heliconius melpomene* (Jiggins *et al.* 2001). It is wisest to conclude: 2→2.

The situation with *Papilio hospiton* and *P. machaon* is clearer, but fits less easily with theory. Obviously these two constitute 'good' species, conforming to morphological, biological and cladistic concepts. *Parnassius apollo* and *phoebus* are a similar case. However, the evidence for some mutual introgression corresponds more closely to the 'genic view' of speciation. Meanwhile, the asymmetrical character of introgression in *Papilio* fits less perfectly. It seems likely that these *Papilio* diverged beyond the point of no return in allopatry, and that introgression occurred only after *P. machaon* again became sympatric. The case of *Parnassius apollo* and *P. phoebus* is similar, but the two species seem likely to have been in close proximity for a long time. In this case, gene flow would have been progressively reduced. Yet, in spite of introgression, all four species remain 'good', in the sense of 'distinguishably different', wherever they overlap.

With the brown *Agrodiaetus*, the situation changes. Hybrids are morphologically undetectable. Karyotype becomes questionable, here, as a species criterion, unless one allows the concept of karyospecies (e.g. Wiemers 2003). Until recently, a karyotype markedly different, either in number or size of chromosomes, was taken as proof of species status because chromosomal differences directly provide mixiological incompatibility. On this basis, allopatric populations distinct in chromosome number were separated as 'good' species. However, frustratingly, Wiemers (2003) and Kandul *et al.* (2004) showed that karyotype variation in this group is sometimes associated with genetic and phylogenetic differentiation, and sometimes not. So how many 'species' are included in Western taxa of brown *Agrodiaetus*? Clearly, *A. ripartii* and *fabressei*, which occur in sympatry, must be distinct (ironically, they have the same chromosome number, but the karyotypes have different morphology). For the other populations, all allopatric and with very variable chromosome numbers, the question makes little sense. Nonetheless, in his excellent, exhaustive work on *Agrodiaetus* and related genera, Wiemers (2003) firmly comes down on the side of all of the other taxa being separate species.

In *Hipparchia*, it seems clear that the best solution is to ignore the more extreme splitters and adopt a moderate lumper approach (Cesaroni *et al.* 1994), but this remains somewhat arbitrary and, again, depends heavily on the status of allopatric units.

The situation observed today in the *Erebia tyndarus* group is typical of the present state of systematics. Taxonomic decisions made during the first half of the twentieth century lacked much biological insight, but the important contribution of genitalic morphology boosted knowledge. After Huxley's 'new systematics', even those specializing in morphology, like Warren, began to take the BSC into account, especially with respect to cohabitation, but also because genitalic differences were assumed to cause mechanical incompatibility during mating. The bulk of progress on the group was, however, made during the 1950s using karyology, in this case a highly efficient tool. Differences between chromosomal morphs are regularly associated with sterility and other deleterious side-effects of hybridization. However, morphometrics, research on contact zones and laboratory crosses were combined with chromosomal studies in a synthetic approach which continues to elicit admiration. It is worth noting the enormous contribution made by de Lesse & Lorković in this field. Access to most populations required ascending many hundreds of metres on foot. In his synthesis, de Lesse (1960a) provided impressive distribution maps. But while data on the most important contact zones and centres of distribution were published in detail, many distributional data accumulated by de Lesse remained unpublished, and were lost when he died.

Mostly, the polytypic or 'biological' species concept was employed. However, a number of pockets of resistance rebelled against any attempt at consensus. The *Erebia tyndarus* and the forms of the *cassioides–arvernensis* complex remain the most contentious. At present, it is clear that the Grindelwald contact forms a 'bimodal hybrid zone' (Jiggins & Mallet 2000). Gene flow might help to explain contradictions between allozyme and mtDNA sequence data elsewhere (Lattes *et al.* 1994, Martin *et al.* 2002). There are large allozyme distances between *nivalis* and the other taxa, and rather slight ones with mtDNA. Indeed, *nivalis* is more of a high-elevation species that must experience a markedly different thermal environment. Watt (2003) has demonstrated that 'differentiation or uniformity of polymorphic genotype frequencies over space may be driven by strong local selection pressures'; allozyme divergence may not always yield results independent of selection.

What was the contribution of molecular markers to improve species delimitation in the *tyndarus* group? Lattes *et al.* (1994) used Nei's genetic distance to separate *cassioides* from *arvernensis* and *hispania* from *rondoui*, but ignored the larger differences between the two populations of *ottomana*, without any particular justification. The main problem of using genetic distance as a criterion of species is that the threshold level may differ in each group studied (Avise 1994). Finally attempts to determine the status of allopatric taxa (including experimental crosses) are rather like division by zero, the cohabitation criterion acting like the denominator that does not exist.

More significant was the much greater utility of molecular data for reconstructing phylogeny distinguishing monophyly from paraphyly. However, a phylogenetic species concept may be difficult to apply in this case. For example, in the tree published in Fig. 4 of Martin *et al.* (2002), *calcaria* and *nivalis* cluster within a group consisting of all the *arvernensis* samples, and together form the sister group to the monophyletic *tyndarus* assemblages. Yet *tyndarus* and *arvernensis* act as separate species, since they meet at a bimodal hybrid zone; this causes a logical anomaly for phylogenetic species, since more basal taxa do not seem to reach species rank, but form a paraphyletic group as far as sexual isolation is concerned (if sexual isolation is considered an apomorphy). Further research will perhaps help to resolve some of the tantalizing questions in this group, but, at present, we must confess an inability to answer precisely the question 'how many species are there?' One can propose a spectrum of solutions spanning two extremes: the 'lumper's' position, with *ottomana, hispania, tyndarus*; or the 'splitter's' position, with the various, very disjunct strains of *ottomana* as 'species', *hispania, rondoui, arvernensis, cassioides, tyndarus, calcaria* and *nivalis*. However, the precise decision along this spectrum will always be more or less arbitrary.

Although also complex, the *Lysandra coridon* group case is somewhat clearer. In particular, if the phylogenetic species concept is capable of wreaking havoc on the *Erebia tyndarus* group, Wu's (2001) 'genic view of species' aids in understanding puzzling features of the *coridon* group. We have mentioned the low level of allozyme differentiation within and between the species of this group, while habitus and ecological features yield stronger, better-supported patterns. One must keep in mind that chromosome number is very high in *Lysandra*. Therefore, each linkage group should be small and, hence, hitch-hiking will affect fewer loci during speciation. A majority of the genome might therefore be exchanged freely, while only regions linked to genes affecting sexual isolation and ecological specialization will be kept distinct by strong selection. Otherwise, in this group, the problem of characterizing species is relatively soluble, provided one cuts some Gordian knots. One example of such a unit is provided by *Lysandra coridon*, which displays a very 'open' population structure, with few if any genetic differences even between geographically distant populations (Lelièvre 1992, Schmitt *et al.* 2002). The main problems are the isolates at the southern periphery of its distribution: *caelestissima* in the mountains of central Spain and *nufrellensis–gennargenti* in Corsica and Sardinia. The stumbling block of the absence of cohabitation is again encountered. By far the simplest and most sensible solution based on such data would seem to be to merge all the forms into a single species, *coridon*, with some strong peripheral subspecies. Likewise, the *albicans–hispana–semperi* complex is best considered a single species with some variation in chromosome number (as in *coridon*) and adaptive features such as voltinism, in the absence of a clear indication from hybrid zones. On the contrary, the frequent occurrence of bimodal hybrid zones between populations of the *coridon* unit, as previously defined, and of members of the *albicans* complex precludes merging them into a single 'good' – or even 'bad' – species unit. This case, in common with the *Erebia tyndarus* group, demonstrates the phenomenon of local mutual exclusion due to similar ecological niches, especially foodplant choice. The criteria of voltinism and chromosome number, ranked highly by de Lesse, proved not much more reliable than other criteria. Therefore, to the question: 'how many species?', we finally answer 'two only' – a simple answer which unfortunately might fray the tempers of some lepidopterists.

CONCLUSIONS

'I have just been comparing definitions of species… It is really laughable to see what different ideas are prominent in various naturalists' minds, when they speak of "species". In some resemblance is everything & descent of little weight – in some resemblance seems to go for nothing & Creation the reigning idea – in some descent is the key – in some sterility an unfailing test, with others not worth a farthing. It all comes, I believe, from trying to define the undefinable' (Darwin 1856). Darwin would have found it even more laughable today: Mayden (1997) enumerated no fewer than 24 species concepts, most of them recent.

Whether species are material, 'real' objects, that exist in the absence of human observers as no other taxonomic rank does, or whether they are only a construction of our mind, is a philosophical problem beyond the scope of this chapter. Our aim is to use the totality of the existing evidence to suggest simple, practical solutions to taxonomic problems, and we attempt to avoid further adding to the vast slag-heap of useless concepts and definitions of the indefinable. Darwin used only a loose definition of species but he was an experienced taxonomist, knew a great deal about describing actual species, and it was sufficient to convince his readership of transpecific evolution. We believe that, even today, a pragmatic, taxonomic solution is more productive than attempting to decide whose concept is correct.

Two facts are undeniable:

(1) Taxonomic decisions based on biological or polytypic species concepts are still common. For instance, Kandul *et al.* (2004) use the term species to mean reproductively isolated populations. Many groups of organisms considered species are well behaved and obey not only the BSC, but also *most* definitions of species.

(2) However, a significant number of rakish taxa will probably always fail to conform to this species morality. They regularly conduct extramarital affairs and produce illegitimate offspring beyond the boundary of the species.

Rogue taxa such as these are the subject of the present chapter. Perhaps the most surprising conclusion we reach is that, in spite of increasing evidence from these well-known European taxa, in some cases flooding out of multiple laboratories using the most modern techniques, many 'bad' species stubbornly remain bad under a variety of species concepts. The existence of such rogues is of course a necessary outcome of gradual Darwinian evolution, and it shouldn't worry us. However, when it comes to placing specimens in drawers or data against a name, bad species are a problem. Unfortunately, constructing a perfect species definition that covers both well-behaved and bad species will almost certainly remain a matter of compromise.

Bernardi (1980) has shown that many a specialist in a given group has tinkered with his own special taxonomic categories to cover this kind of situation. An example is the 'semispecies' idea of Lorković & Mayr, but many other examples are scattered throughout the obscure or forgotten literature. Is the solution to house rogue taxa in a special fuzzy species ghetto? This might have been a good idea if bad species were a homogeneous group; however, as we have seen, the intermediate states are variable. In any case, there is no agreement today about the rank even of the supposedly most objective of taxa, the species itself (Isaac *et al.* 2004). We thus argue that classical taxonomic ranks – species and subspecies – are all we require, to avoid proliferation of ever more finely divided categories.

Returning to the actual bad species analysed above, let us ignore problem taxa that result from taxonomic error, such as the undetected 'good' species *Leptidea reali* or the hybrid *Erebia* '*serotina*'. In the case of *Zerynthia*, there is intraspecies incompatibility, coupled with interspecies compatibility; this was discovered only through artificial crosses of geographically separate populations. Perhaps, therefore, we should proclaim the primacy of observations in natural contact or cohabitation over experimental tests, which can give an inaccurate impression of pre- and postzygotic compatibility (Mayr 1963, Mallet 1995). If geographically and genetically intermediate populations disappear, for some reason, we end up with the problem of allopatric entities (see below). Sometimes divergence is so great that it seems logical to classify allopatric taxa as species. But is it really necessary to consider continental and British strains of *Lasiommata megera* as different species because they display some genetic incompatibility (Oliver 1972)? We argue it is more informative not to do so.

In the three papilionids (*Zerynthia, Parnassius, Papilio*), most people looking at natural populations in zones of overlap would declare each pair of species to be 'good', even when hybridization occurs regularly, but sparsely, in at least some areas of cohabitation. We suggest that the same decision should apply to all other cases of bimodal phenotypic and genotypic distribution where hybrids occur (Jiggins & Mallet 2000), whether or not actual or potential gene flow (introgression) takes place. Similar decisions may be made without difficulty for parapatric species with a contact zone and limited or exceptional hybridization as in the *Erebia tyndarus* group. In the case of *Lysandra*, *Pontia daplidice* and *edusa*, and probably *Melanargia galathea* and *lachesis*, the presence of a bimodal hybrid zone allows us to consider the taxa in contact as species, but here we are near the boundary condition, because, if hybridization becomes much more frequent, hybrid swarms would result, and overlapping populations would become merged into a single, unimodal population. For *Pontia*, there are divergent opinions: Geiger *et al.* (1988) and Wenger *et al.* (1993) consider *daplidice* and *edusa* as (semi-)species, while Porter *et al.* (1997) grant them only subspecies rank.

Allopatric forms separated by major geographic discontinuities give rise to a virtually insoluble difficulty. Here, there is a Gordian knot to cut. Mayr (1942, 1963, 1982) repeatedly justified the BSC as the only 'non-arbitrary definition of species', but even he (1982: 282) admits 'the decision whether to call such [allopatric] populations species is somewhat arbitrary'. Sperling (2003) likewise suggested that decisions should be made using information, such as genetic distance or karyotype, from closely related taxa that are in contact. This is essentially already implicit in the argument for the use of 'potential' gene flow in the BSC. An absolute threshold of similarity or distance is arbitrary, so no one should harbour illusions about the 'reality' of species delimited by this pragmatic approach. The most important objective is to preserve clarity, parsimony and stability in nomenclature. Therefore, endemics on Tyrrhenian or Atlantic islands might often be considered subspecies of mainland species if they are moderately differentiated, and we argue that this solution should be employed as far as possible on parsimony grounds. They should be considered species only if they present clear signs of very strong genetic, morphological and biological differentiation above that expected of related mainland species in contact with close relatives. When it comes to allopatric 'karyospecies', one might wish to follow Wiemers (2003), and give specific rank (especially if strongly divergent at other genetic markers). Even here, use of the same species name with chromosome number placed in parentheses would be as informative; this is typically applied, for example, in *Mus musculus*. In general, decisions about the species status of allopatric neighbours is always somewhat arbitrary, and a lot less interesting than obtaining field or genetic data from hybrid zones and parapatric contact zones, or from unimodal lines. Here, one deals with a concrete phenomenon, rather than an investigation into how many angels fit on the head of a pin.

We therefore argue for revival and a modern, scientific justification of the rather neglected and misused (and perhaps rightly, in many cases, much-maligned) rank of subspecies. Very often, subspecies have been used to describe geographical forms recognizable only to their author, which has led to disrepute. But today there is a refreshing trend among lepidopterists to consider only more strongly distinct forms (in morphology, ecology or genetics) as subspecies, and to lump more dubious geographical forms as synonyms. These general recommendations provide a useful compromise between description of geographical variation, the needs of modern butterfly taxonomy (for example, see Ehrlich & Murphy 1984, Sperling 2003), and Darwin's pragmatic use of the term species in evolutionary studies.

It is a Sisyphean task to try to give a definitive, irrefutable definition of species, but species will continue to function as useful tools in biology for a long time. To the question raised by the French population geneticist Le Guyader (2002): 'Must we give up on a species concept?' we answer: 'No!' We recommend that researchers of the future study gene exchange in the many hierarchical layers of phenotype, genotype and genome in 'bad' species of butterflies. This has been done in only a handful of species, such as the larch bud moth (Emelianov *et al.* 2004). Such studies will be surely much more illuminating about the nature of speciation and evolution at the species level than endless discussions on the 'essence' of species.

APPENDIX: TOOLS FOR TAXONOMIC PRACTICE AT SPECIES LEVEL IN BUTTERFLIES

The previous parts of this work presented first the theoretical background of taxonomic work on species, and then a series of analyses of peculiar real cases. To sum up, species are delimited by a series of criteria derived from the concept used and the speciation theory associated with it, with an accent on studies on populations in cohabitation or contact.

There are many different types of datasets that can be used. Wing colour morphology is perhaps the most obvious, and of course in butterflies is extremely important. Ecological, behavioural and distributional data are also important. Differences in genitalia have often been considered to be significant for reproductive isolation via a 'lock-and-key' hypothesis (Jordan 1896, Porter & Shapiro 1990). As already seen, genitalic data are useful in certain cases, but not always. Chromosomal data are more often reliable, but they can also be misleading. The same might also be true for pheromonal characters, which can be considered both as organismic and synepigonic, but there is little information on butterflies (but see Andersson *et al.* 2003).

We here present an overview of currently available methods for gathering and analysing taxonomic data and conducting biological and statistical studies to establish whether taxa might be species or taxa at some subspecific category. Nomenclatural aspects of species delimitation, however, do not form part of the remit of our chapter.

Morphological characters

Data acquisition

Empirical and intuitive, qualitative observations are still used, but biometrical methods have become more normal. Even with qualitative characters, records of a series of states are often performed. In adults, the hard parts of the exoskeleton are most often studied, and genitalia have remained favourite characters since the late nineteenth century (Jordan 1896). Wing-pattern variation is used in butterflies predominantly because it is both evolutionarily labile and easy to detect and score, and provides useful data for identification in most cases. A still commonly used method in morphometrics consists of measuring anatomical structures under a microscope with a micrometer (see e.g. de Lesse 1960a, Cesaroni *et al.* 1994). Today, automated measurements employing digital imaging can also be used. Larval characters can also be useful: superficial features (pigmentation, pattern) are commonly used, but chaetotaxy of first-instar larvae sometimes provides very significant information. The microstructure of the eggs is a great favourite, especially using scanning electron microscopy (SEM). In using egg sculpturings, one must remember that it is actually an imaginal feature, since it results from the imprint of ovary follicles.

Data analysis

Analysis of morphological data may be performed character by character. It is also possible to integrate a dataset from a sample of individuals in multivariate, or reduced space, analyses (RSA). These methods have been great favourites for the French school of statisticians, long led by Benzecri. Systematists may sometimes be reluctant to use them, but they are powerful when correctly used. The reader should consult works such as Sneath & Sokal (1973) for details of clustering and ordination methods. In brief, there are three main categories of RSA: principal components analysis, using Euclidean distance, factorial correspondence analysis, using a chi-square-based distance, and factorial discriminant analysis (FDA). The latter seems to be the most appropriate to conduct a study on a dataset that may reasonably be supposed to include two (or more) different species. A frequent criticism of RSA is that these methods are descriptive, rather than inferential statistics. However, with some practice, they are excellent tools for exploring a dataset. Genetic data can also be analysed in the same way.

Chromosome characters

The study of chromosomes in butterflies was for a long time dominated by the work of Lorković (1941) and de Lesse (1960a). Since that time, interest has moved towards other types of genetic markers, but chromosome studies are still useful (e.g. Munguira *et al.* 1994, Wiemers 2003). Chromosome counting is typically practised on meiotic cells in the testes during spermatogenesis. Generally rounded, small and numerous, lepidopteran chromosomes are not gratifying objects of study. In approximately 1000 species of Lepidoptera, the distribution of chromosome numbers proved markedly leptokurtic and asymmetrical, with a strong concentration around the modal number ($n = 31$), and an extreme scattering of frequencies for the higher numbers (Robinson 1971). Some members of *Polyommatus* (*Plebicula)* (Lycaenidae) display the highest chromosome numbers in metazoans (190–191 for *P. nivescens*, and for *P. atlantica*), while numbers less than 10 are observed in *Erebia* (de Lesse 1960a). Supernumerary chromosomes are often seen, especially in Satyridae and Hesperiidae, and may produce pronounced intraspecific variation, in particular in *Plebicula* (de Lesse 1960a).

The significance of chromosome number variation in butterflies has been widely debated (Lorković 1941, Robinson 1971, White 1973, Kandul *et al.* 2004). Polyploidy seems unlikely as a general mechanism for chromosome number variation in butterflies, despite Lorković's (1941) views. Centromeric fusion or fission seems a more probable cause of chromosomal number variation (Suomalainen 1965, White 1973, King 1993). This could be due to the structure of the lepidopteran centromere, which is reportedly 'diffuse' (Federley 1945, Suomalainen 1953; but see Gus *et al.* 1983). A diffuse centromere may allow some amelioration of damage suffered in chromosomal heterozygotes during meiosis. Another insect group with diffuse centromeres, scale insects, also show large variation in chromosome numbers (Cook 2000). On the other hand, the modality of chromosome number around 31 throughout the Lepidoptera is not easily accounted for under this scenario (White 1973). Kandul *et al.* (2004) suggest that instances of enhanced chromosome number variation could result from epidemics of transposable genetic elements.

In practice, chromosome study in butterflies is tedious because spermatogenesis often terminates early in adult life. Even in young males, meiotic metaphase equatorial plates in the spermatids, the most favourable stage for counting, are usually scarce. In addition, chromosomes are usually so highly condensed that little intrachromosomal structure is visible. However, particularly in *Polyommatus* (*Agrodiaetus)*, differentiation of larger, so-called macro-chromosomes which vary in number and size has been found useful (de Lesse 1960b, Munguira *et al.* 1994, Lukhtanov & Dantchenko 2002b). Moreover, instead of producing conveniently visible giant polytene chromosomes as in Diptera, Lepidoptera appear to adopt polyploidy as a means of up-regulating gene expression in highly active somatic tissues – far less easy to use as a taxonomic or genetic marker.

Hesselbarth *et al.* (1995) put forward the hypothesis that chromosome fission and fusion could have an influence on adaptive abilities. Species with low chromosome numbers should be associated with greater genome stability and more supergenic association and therefore adapted to stable environments. Conversely, high chromosome numbers should ease recombination and generate many genotypes promoting adaptation to new or unstable environmental conditions. Wiemers (2003) found absolutely no evidence of such a phenomenon in *Agrodiaetus*, the genus displaying the largest variation in chromosome numbers in butterflies. We suggest another possible effect of high chromosome numbers: by increasing the average rate of recombination, they could limit hitch-hiking of genes causing incompatibility and could therefore ease introgression of 'neutral' genes in hybrid belts (e.g. in *Lysandra*).

Karyotypic differences between taxa are often taken as a proof of species-level distinction, and this argument can be legitimate. However, caution must be exercised. Supernumerary, genetically insignificant B-chromosomes are common (de Lesse 1960a, 1961b), and might sometimes be an indication of hybridization (Wiemers 1998); moreover, when morphologically and ecologically very similar groups of populations occurring in different areas display different karyotypes, it may be premature to base species separation on chromosomal number, in the absence of other evidence such as molecular studies. The term 'chromosome races' (Goldschmidt 1932) does not seem to have been used explicitly in butterflies, but de Lesse (1966) maintained, within a single species, allopatric populations of *Agrodiaetus dolus* from southern Europe with $n = 108$, 122 and 124; in contrast, Munguira *et al.* (1994) split the taxa into separate species with different karyotypes. Experiments carried out in moths of the genus *Antheraea* showed that two 'species', *A. roylei* and *pernyi* with $n = 18$ and 49 respectively, could be intercrossed for 32 generations with fertility and viability intact (Nagaraju & Jolly 1986).

Molecular characters

The history of molecular systematics can be divided into two major stages: a protein phase and a DNA phase. The former, based mainly on allozyme electrophoresis, became important at the end of the 1960s with studies on *Drosophila* and humans (Avise 1974, Richardson *et al.* 1986, Hillis *et al.* 1996), and played a major role in butterfly systematics from the 1970s onwards (Geiger 1990). The DNA phase really came into its own in the 1990s following the development of the polymerase chain reaction (PCR).

Protein data

Since the earliest days, electrophoretic study of protein polymorphism revealed a stunning amount of variation (Lewontin 1974). A bitter debate on the significance of these observations took place in the 1960s and 1970s: some championed selection as a cause for polymorphism, while others raised mathematical objections (Kimura 1968) and argued that it must be neutral. Current experimental (Watt 2003) and theoretical (Gillespie 1991) evidence suggests that both selection and neutral evolution may be important; consequently, when using protein variation to study taxonomic units, one must be careful that selected variation affecting ecological parameters, such as foodplants (Feder *et al.* 1997), does not obscure taxonomic conclusions.

Analysis of protein data

A classical method for analysing allozyme data is to reduce the multilocus data by means of a calculation of overall genetic distance (Hillis *et al.* 1996). This can be used in cluster analyses, and subsequently to phylogenetic inference, but there is no obvious level of genetic distance above which two samples can be confidently considered to be separate species. Nei's (1978) genetic identity (I) and distance ($D = -\ln I$) is regarded as particularly useful, because it corrects for small sample size and for multiple 'hits', and so should be proportional to time since divergence under a molecular clock. Closely related species of *Drosophila* may be in the range of Nei's D of 0.05–0.50 or so (Coyne & Orr 1997). In European butterflies, the genetic distances between species of the same genus range generally between 0.05 and 0.15 (Aubert *et al.* 1996b, Geiger 1990, Zimmermann *et al.* 1999). However, pairs of apparently closely related species may be more distant, and, more surprisingly, other pairs of species may coexist without hybridizing, but differ hardly at allozyme loci ($D < 0.01$). Diagnostic loci (fixed for different alleles in each population) are useful for studying hybridization and gene flow between taxa.

Allozyme studies within species have often attempted to estimate gene flow based on the neutral expectation of gene frequency variation. Firstly, one may estimate the variation of gene frequencies between populations via the use of F_{ST}, the standardized variance of gene frequencies, which measures the fraction of genetic diversity (heterozygosity, H_e) found between populations. If gene frequency variation can be assumed to be a balance between homogenization via gene flow (m) and local divergence due to genetic drift (proportional to $1/2N_e$, where N_e is the effective population size), then $F_{ST} \approx 1/(1 + 4Nm)$. However, there are many problems with these methods, which allow the estimation only of the combined parameter $N_e m$. They should not be applied in any context other than under equilibrium between genetic drift and gene flow; it does not, for instance, apply in the case of gene flow and hybridization between two species, or between ecologically differentiated taxa (Mallet 2001), because here selection will be involved in the differentiation (*contra* Porter & Geiger 1995). In such cases, strong natural selection may lead to rapid equilibration of gene frequencies in the presence of gene flow. A much more useful method is available based on correlations (or linkage disequilibria) between loci diagnostic or with strong frequency differences between hybridizing taxa. Hybrid zones, in particular, allow estimation of selection and gene flow separately (Mallet *et al.* 1990, Porter *et al.* 1997, Mallet 2001, Blum 2002, Dasmahapatra *et al.* 2002).

A species criterion based on 'genotype clusters' (Mallet 1995) can be viewed as an extension of this multilocus method. Genotypes reach bimodality only when several characters or loci are in tight linkage disequilibrium. One may use 'assignment methods', likelihood or distance-based multivariate statistics (see above under 'Morphological characters') to cluster genotypes, to determine whether multilocus gaps between clusters are significant; if so, the clusters can be classified as separate species (Aubert *et al.* 1997, Feder *et al.* 1997, Deschamps-Cottin *et al.* 2000). Newer likelihood or Bayesian methods also allow estimation of the rates of hybridization in a sample of a pair of several, bimodally distributed taxa (Cianchi *et al.* 2003, Emelianov *et al.* 2003, 2004).

DNA data

DNA methods have outstripped allozyme electrophoresis, but are still in their infancy compared with what might be possible in a few years. The mitochondrial genome, with a mere 16 000 base pairs, has been far the most widely used in butterflies (Pashley & Ke 1992, Wahlberg & Zimmermann 2000), and elsewhere. Intraspecific mtDNA sequence polymorphism occurs in certain butterfly species but is absent in other cases. For instance, *Papilio machaon* displays polymorphism throughout its range (F. Michel, pers. comm.), as do *Euphydryas aurinia* and *Melitaea athalia* (Zimmermann *et al.* 2000). In contrast, no variation within *Euphydryas maturna* has been observed across a large range

(Zimmermann *et al.* 2000). The mitochondrial genome is very sensitive to genetic drift, since it has a N_e four times smaller than that of the nuclear genome. Comparison between closely related species usually shows 1–2% divergence, but strikingly low differences are observed in some instances: 0.2 % between *Euphydryas maturna* and *E. intermedia*. We therefore do not believe that any particular level of divergence can be used as a suitable benchmark or 'DNA barcode' for species status.

Nuclear gene sequences are beginning to be used with some success (e.g. Brower & Egan 1997, Beltrán *et al.* 2002), while microsatellite loci have proved disappointingly difficult to obtain in butterflies (Nève & Meglécz 2000, Meglécz *et al.* 2004). Amplified fragment length polymorphisms (AFLPs) can also be used as a very abundant source of 'fingerprint' markers in analyses of natural populations, including studies of hybridization in nature (Emelianov *et al.* 2004). Nonetheless, while useful in mapping, AFLPs are relatively untried as tools for studying populations.

In summary, marker data, whether morphological, cytological or molecular, have allowed us to search organisms for characters with increasing thoroughness, but are not fundamentally different from one another.

Ethological and ecological criteria

Treating ethological and ecological characters together seems hardly justified, since they are heterogeneous. However, they all play an active role both in cohesion *within* species and in maintaining separateness between species. They therefore give access to the very factor, reproductive isolation, important in speciation. We will consider the following most important categories. Firstly, there is the ecological niche and its main constituents: habitat and foodplant choice, phenology and diapause; secondly, sexual behaviour and pheromones; and thirdly, geographical distribution, particularly cohabitation.

According to Gause's principle (1934), if two species occupy the same niche, they will mutually exclude one another and will display parapatric distributions, with very limited cohabitation. These cohabitation zones may not necessarily imply hybridization and/or genetic proximity. Alternatively, if the two species share a large area of sympatry, they must be ecologically differentiated. The main difficulty in using such ecological information is circularity. Very often, field entomologists 'feel' that two putative species display subtle differences in habitat use but are unable to develop inferential tests to support their impression. Various parameters of the ecological niches occupied by butterflies frequently crop up in studies of butterfly species.

Larval foodplant choice

The host plant is perhaps the key niche dimension in the life of a phytophagous insect (Dethier 1954, Futuyma & Keese 1992, Feeny 1995, Berenbaum 1995), and feeding regime may play a major role in speciation, including in some Lepidoptera (Feder 1998, Drès & Mallet 2002). Butterflies are generally oligophagous and change in diet is likely to result in a selective regime that might lead to speciation and adaptive radiation (Ehrlich & Raven 1964). There is certainly evidence for rapid diet evolution in some taxa, such as the Papilionini (Aubert *et al.* 1999) or Melitaeini (Mazel 1982, Singer *et al.* 1992a). These changes may appear spectacular, with switching between plant families common (e.g. Rutaceae to Apiaceae in *Papilio*, Dipsacaeae to Caprifoliaceae and Valerianaceae in *Euphydryas*); however, these unrelated plants almost always have important chemical similarities (Bowers 1983, Berenbaum 1995). The evidence for host-related speciation in butterflies is thus somewhat weak (see e.g. Nice & Shapiro 2001, for a case in the Lycaenidae). In the North American *Euphydryas*, where rapid intraspecific diet evolution has been observed, new host adaptations normally evolve rapidly in local populations, and drive original preferences and adaptations to extinction, rather than causing speciation (Thomas & Singer 1998).

Diapause control and voltinism

A butterfly population is expected to have as many broods as climatic conditions and food availability allow. Intraspecific variation in voltinism is common in species that have a wide range. *Melitaea athalia*, for example, is univoltine in northern Europe, bivoltine in warm regions with a wet summer, and univoltine again in the Mediterranean and in mountains above 1000 m (see also the *Papilio* paragraph above). On the other hand, sometimes it forms a character presumed to differ at the species level: for example the species *Aricia artaxerxes* is univoltine and occurs in northern Europe, but is replaced by the bi- or multivoltine species *A. agestis* in southern Europe. Univoltinism can also be a constitutive character within a taxon: in the genus *Euphydryas*, for example, all species are single-brooded, even under conditions that could allow several broods.

The genetic determination of diapause in Lepidoptera has been studied in few cases, where it apparently involves a number of interacting loci (Held & Spieth 1999); in other cases, it appears to give a pattern suggesting sex-linked inheritance and few genetic factors. In some cases, crosses between related subspecies or species in the Papilionidae give classic 'Haldane's rule' asymmetry in diapause between males and females, suggesting the importance of Z-linkage (see the *Papilio* section in this chapter and below in this part).

Mixiological criteria

'Mixiological' is the term applied, especially in France, to phenotypic and behavioural traits which affect hybridization and introgression between pairs of taxa. In spite of a heated debate about the use of terms such as 'isolating mechanisms' (Lambert *et al.* 1987, Mallet 1995), all sides agree that a restriction of gene flow is the key process in speciation in sexual taxa. Since many factors may produce this result, it is normal to aggregate these heterogeneous traits under the same heading, 'reproductive isolation' (Mayr 1963); the two major kinds of reproductive isolation are prezygotic and postzygotic isolation.

Prezygotic barriers

These may involve spatial and temporal isolation (habitat choice and phenology), mating behaviour and courtship, pheromone differences, mechanical barriers to pairing, and physiological features of insemination before gametic fusion. Prevailing opinion about their origin is that prezygotic barriers are often formed as a by-product of intraspecific coevolution, with selection maintaining compatibility (as in the 'recognition' concept of Paterson 1985) while the system of mating or reproduction diverges. Another argument is that selection may cause divergence in pre-mating traits as a directly selected process ('reinforcement') to avoid the production of unfit hybrids. Reinforcement has been much debated (Paterson 1985, Lambert *et al.* 1987); however, the phenomenon has been demonstrated in some cases (e.g. Noor 1995, Lukhtanov *et al.* 2005), and is suspected in the tropical genus *Heliconius* (Jiggins *et al.* 2001).

Postzygotic barriers

These involve inviability or sterility acting on hybrids from the zygote stage onwards. Hybridization experiments show that hybrids between species are often inviable or sterile. Sterility was demonstrated, for example, using *Drosophila* as a research material (Dobzhansky 1937), but hybrid sterility had been recognized as early as Buffon's time (Mayr 1982). However, hybrid sterility and inviability between taxa considered 'good species' is far from general (Darwin 1859, and several examples in the present chapter). Fitness is often reduced in hybrids (Rice & Hostert 1994), not only in physiology (intrinsic or endogenous selection) but also in ecological adaptations that allow individuals to exploit niches of parental taxa (extrinsic or exogenous selection) (Hatfield 1996, Jiggins & Mallet 2000). Crosses in captivity must be considered with utmost caution, since careful rearing and pampering can allow certain experimentally obtained hybrids to survive, while they would undoubtedly die under natural conditions. Conversely, the diseases associated with captivity and promiscuity, or unsuitable breeding conditions, can cause the loss of broods which could have thrived in the wild. This uncertainty allowed such wags as Loeliger & Karrer (2000) to cast doubt on earlier results of Clarke & Sheppard (1953, 1955, 1956) and Aubert *et al.* (1997), and to negate the existence of postzygotic incompatibilities between *Papilio machaon* and *P. hospiton* – an extraordinary assertion contradicted by all the evidence!

It has become de rigueur to refer to all kinds of hybrid inviability and sterility as Dobzhansky–Muller incompatibilities (Orr 1995), given that they rarely cause inviability or sterility within species, but only when transferred to another genetic background; in other words, their evil effects result from epistatic incompatibilities between genes. It is likely that hybrid inviability between populations can evolve, paradoxically, without producing fitness problems within populations at any time during its emergence.

Let us finish this work by looking more closely to a striking type of genomic incompatibility we have frequently evoked: Haldane's rule (Haldane 1922): in hybrids, the heterogametic sex (the one with heterogeneous sex chromosomes, e.g. XY) tends to be more sterile or unviable than the homogametic sex (e.g. XX). The heterogametic sex is the male in most insects, including *Drosophila*, as well as in mammals. The Lepidoptera and birds are notorious exceptions, having heterogametic females: their sex chromosome formula is ZW in the females and ZZ in males, yet obedience to Haldane's rule in Lepidoptera is as good as or better, in reversed form, as in the species with XX/XY sex-determination (Presgraves 2002). It is surprising, perhaps, that agreement on the explanation, 'dominance theory', of the striking facts of Haldane's rule has been reached only recently: the earliest loci to diverge appear to cause incompatibilities only recessively; thus incompatibilities tend to affect the sex chromosome, and mainly in the heterogametic sex. In agreement with dominance theory, sex-linkage of incompatibilities holds for butterflies, where the female is most strongly affected (Grula & Taylor 1980, Sperling *et al.* 1990, Aubert *et al.* 1997, Jiggins *et al.* 2001, Naisbit *et al.* 2002) as well as for *Drosophila*, where it is the male (Coyne & Orr 1997). It is interesting that the general applicability of Haldane's rule in the Lepidoptera (Presgraves 2002) implies that maternally inherited markers, such as mitochondrial DNA or W-chromosomes, will rarely be transmitted between species (Sperling 1990). Thus, species identification based on mitochondrial 'DNA barcodes' may work better for Lepidoptera (Hebert *et al.* 2003) than in other taxa prone to hybridization and introgression.