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The effect of habitat type on speciation rates and range movements in aquatic beetles: inferences from species-level phylogenies

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Abstract

Most aquatic beetles in the family Dytiscidae are tightly associated either with running (lotic) or stagnant (lentic) water bodies. The range size of lotic species is known to be, on average, much smaller than that of lentic species, presumably as a result of differences in dispersal strategies in each habitat type. We explored possible effects of these differences on clade evolution and speciation rates by comparing species-level phylogenies based on cytochrome oxidase I (COI) and 16S rRNA mitochondrial genes for two genera, the lentic *Ilybius* and the lotic *Deronectes*. The expectation that species turnover is higher in lotic lineages due to their lower dispersal propensity compared to lentic species was not strongly supported. *Deronectes* displays a higher frequency of recent splits than *Ilybius*, consistent with the hypothesis, but the difference was not significant compared to expected patterns under a constant speciation rate null model. Similarly, when the degree of sympatry was plotted against relative node age, more allopatric splits were evident in the lentic *Deronectes*, suggesting a slower rate of range movement since speciation, but the differences were not significant. We discuss two explanations for our failure to detect differences between the two clades. First, current methods for analysing species-level phylogenies may be sensitive to taxonomic and sampling artefacts. Second, lentic and lotic clades may indeed display similar levels of species turnover despite occupying very different habitats at different spatial scales. More work is needed to investigate the effects of population level processes and spatial scale on macroevolutionary dynamics.

Keywords: allopatric speciation, Dytiscidae, habitat associations, lotic–lentic differences, lineage-through-time plots, mtDNA

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Introduction

The habitat constrains many aspects of species' ecology (Southwood 1977; Southwood 1988), and affects biogeography and population structure by determining the spatial matrix in which species persist. Habitat associations therefore determine species attributes such as the size and extent of geographical ranges, the local and regional abundance and the level of genetic substructuring. It is expected that these parameters will also influence the rate of speciation, as the spatial arrangement of habitat patches affects the degree of connectivity between populations and their propensity for allopatric speciation. Similarly, habitat parameters can affect the risk of extinction when habitat is short-lived and widely spaced, so that colonization of new habitat patches may be relatively infrequent compared to local population extinction (Hanski & Gyllenberg 1997). Hence, through its effect on the genetic architecture of species, habitat type may also have macroevolutionary implications and influence the number of species in a lineage. Yet, the effect of population level processes on macroevolutionary patterns has not been widely explored.

This may be due to the fact that there are few good study systems for testing the effects of differences in spatial (and genetic) structure on mode and rate of speciation. Freshwater

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ecosystems, in particular small streams and ponds, lend themselves especially well to such study as the spatial matrix of habitat patches represent clearly circumscribed localities in an otherwise diffusely structured landscape. Distances between patches are discrete and can be unequivocally measured. Aquatic habitats impose stringent boundaries on the inhabiting organisms and dispersal between habitat patches requires movement across a different habitat, as the water bodies usually have no direct connections between them. Thus, differences in the spatial arrangement of these habitats might be expected to exert a strong effect on the level of population subdivision and genetic structure.

An ecologically particularly important difference among aquatic habitats is whether they are standing (lentic) or running (lotic). In many aquatic organisms, as in the aquatic beetles investigated in this study, species are confined to either one of these habitat types, with only a small proportion of species occurring in both. This may have to do with the different requirements of species' swimming performance in running and stagnant water bodies, or the indirect effect on the chemical and physical properties of either habitat type. However, both habitats also differ in their temporal persistence, as small ponds tend to fill with sediment over a time period of decades or centuries. In contrast, streams persist over geologically defined periods, even if their exact location may change or if they dry up seasonally. These differences in habitat dynamics can be expected to impact the inhabiting organisms, and to result in different levels of population subdivision and genetic structure.

Previous results have shown an intriguing correlation between habitat type (i.e. either running or stagnant water) and geographical ranges in water beetles (Ribera & Vogler 2000; Ribera *et al*., in preparation). A survey of 400 + species of aquatic Coleoptera of the Iberian Peninsula revealed that a large proportion of lotic species is confined to a small proportion of the major biogeographical provinces of Iberia, whereas lentic species are usually distributed over the entire Peninsula and beyond (Ribera & Vogler 2000). This observation may be indicative of the differences in ecological strategies of both groups: lentic species exist in a dynamic matrix of habitats where individual patches tend to disappear quickly and active dispersal to new habitat is instrumental to the long-term persistence of the population (in agreement with the general observation that species with high dispersal abilities should have wider distributions, e.g. Hanski *et al*. 1993; Gutiérrez & Menéndez 1997). In contrast, lotic populations could persist without such long-distance dispersal, tracking the movements of river systems should their location shift. The spatial structure imposed by the habitat type therefore may result in different ecological strategies.

Here we explore the use of species-level phylogenies to test possible macroevolutionary consequences of these differences in ecology and range size. According to the scenario outlined above, lotic species are expected to display strong geographical structure between habitat patches (river systems), whereas the need for dispersal in lentic species should result in a homogenization of the gene pool throughout a wide geographical range. This fact would limit the potential for allopatric speciation in lentic species, and therefore speciation events should be rare over time. However, as noted, extinction rates are also expected to be lower, due to the higher abundance and wider distribution of individual species, resulting in altogether lower species turnover in lentic vs. lotic species. A comparison of lotic and lentic beetle species presents a unique possibility to investigate the consequences of habitat association on geographical structure and clade evolution.

To test these predictions, we used two species groups of aquatic beetles in the family Dytiscidae, the genus *Deronectes* and the *Ilybius subaeneus* group (the former genus *Ilybius*, Nilsson 2000). The first includes 53 recognized species, with a predominantly Mediterranean distribution reaching central Asia in the east (Fery & Brancucci 1997; Fery & Hosseinie 1998). Species are almost exclusively found in small creeks and rivers, with only two species able to also inhabit stagnant waters. Species are usually restricted to relatively small geographical ranges, frequently in mountain regions. It is the largest clade of Palaearctic Dytiscidae entirely confined to running waters. In contrast, the 33 recognized species of the *Ilybius subaeneus* group occur almost exclusively in stagnant water, and have generally wider geographical ranges throughout large parts of the Palaearctic or Nearctic, with some Holartic species (Larson 1987; Nilsson & Holmen 1995). Together with the genus *Rhantus*, they are the most species-rich clade of the Palaearctic fauna confined to stagnant water.

Based on species-level phylogenies derived from mtDNA sequences we compared apparent diversification rates in the lotic *Deronectes* and lentic *Ilybius*, calibrated to the number of molecular changes. If species turnover were higher in *Deronectes* than *Ilybius*, we would expect a larger proportion of recent nodes in the former clade. We tested for significant differences between the clades compared to expected patterns under a constant speciation rate null model. In addition, assuming a generally allopatric mode of speciation, support for the hypothesis could also be obtained from the amount of range overlap between closely related species. If range movements are high, recently diverged species should be more highly sympatric than under low rates of movement (Berlocher 1998; Barraclough & Vogler 2000). We therefore calculated the amount of range overlap in close relatives throughout the phylogeny in a comparison of *Deronectes* and *Ilybius*. If lentic species are dependent on high dispersal, as hypothesized, the degree of sympatry at the early stages of divergence should be higher than in lotic species.

Materials and methods

Taxon sampling and DNA sequencing

Twenty-three of the 53 known species of *Deronectes* were included in the molecular analysis, selected to obtain a complete representation of the western Mediterranean taxa. The taxon sampling included representatives of all species groups recognized in the revision of Fery & Brancucci (1997), with the exception of the *doriae* group which comprises four species distributed in Greece and Turkey. The *abnormicollis* group, also limited to the eastern Mediterranean, was represented by three species (*D. abnormicollis*, *D. persicus* and *D. youngi*). Among the western Mediterranean groups of species, only one species from Mallorca (*D. brannani* Schaufuss 1869) and two from North Africa (*D. peyerimhoffi* Regimbart 1906 and *D. perrinae* Fery & Brancucci 1997) could not be included. Missing species are morphologically very close to some of the included species (see Fery & Brancucci 1997), and their potential inclusion is unlikely to affect the conclusions about relationships established here. Although a number of subspecies of western European *Deronectes* have been described (Fery & Brancucci 1997; Fery & Hosseinie 1998) these were not separated in the analysis.

The genus *Ilybius* was represented by 19 of the 33 known species of the *Ilybius subaeneus* group, including all European species with the exception of *I. picipes* (Kirby 1934), and several East Palaearctic and Nearctic species (see Table 1 for the ranges of the included species). Appropriate outgroups were selected based on current knowledge (Nilsson & Angus 1992; Nilsson 2000) and a genus-level phylogenetic analysis of Dytiscidae currently in progress (I. Ribera *et al*. unpublished). Specimens used were collected in the field or obtained from colleagues, with details of localities and collectors listed in Table 1. Voucher specimens are kept in the Entomology Department of The Natural History Museum, London.

Total DNA was extracted from single specimens freshly collected into ethanol as described previously (Vogler *et al*. 1993). Only the thorax was used for large beetles, and the entire specimen for small beetles. Sequences of 16S rRNA were amplified in a single fragment of ≈ 500 bp, using primers 16Sa (5′ ATGTTTTTGTTAAACAGGCG) for the 5′ end of the gene and 16Sb (5′ CCGGTCTGAACTCAGATCATGT) for the 3′ end. A single fragment of ≈ 800 bp (for *Deronectes*, corresponding to the amino acids 221–508 in Lunt *et al*. 1996, from the middle of the region E3 to the COOH end) or 700 bp (for *Ilybius*) of cytochrome oxidase I (COI) was amplified using the primers 'Jerry' (5′ CAACATTTATTTTGATTTTTTGG) for the 5′ end of the gene, and 'Pat' (5′ TCCAATGCACTAATCTGCCATATTA) for the 3′ end (Simon *et al*. 1994). All sequences generated in this study were deposited in GenBank (Acc. nos AF309241–AF309353), and aligned data matrices can be obtained from http://www.bio.ic.ac/staff/apvogler/deronectes.htm.

The following cycling conditions were used: 1–2 min at 95 °C; 30 s at 94 °C, 30 s at 47–50 °C (depending on the melting temperatures of the primer pair used), and 1–2 min at 72 °C (repeated for 35–40 cycles); 10 min at 72 °C. Amplification products were purified using a GeneClean II kit (Bio 101, Inc.) Automated DNA sequencing reagents were supplied by Perkin Elmer Applied BioSystems Ltd. (ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit). Sequencing reactions were purified by ethanol precipitation (see the Applied Biosystems product literature) and were electrophoresed on an ABI3700 sequencer. Sequencing errors/ambiguities were edited using the SEQUENCHER 3.0 software package (Gene Codes Corporation).

Phylogenetic analysis and molecular evolution

Sequences for COI were not length variable, and 16S rRNA sequences differed in length only minimally, affecting mostly the outgroup taxa. Alignment therefore was performed manually, by attempting to maximize sequence similarities; tree-based methods that might be more appropriate for sequence and a wider exploration of alignment parameters were not used because of the expected minimal effects. Phylogenetic analysis was performed with PAUP 4.0 (Swofford 1999), using parsimony procedures for tree reconstruction [100 random replicates using the tree bisection reconnection (TBR) branch swapping], and using gaps as a fifth character. Constraint trees for determining Bremer Support values (Bremer 1994) were generated with TREEROT (Sorenson 1996). The significance of the incongruence length difference (ILD) (Farris *et al*. 1994) was assessed with the Partition Homogeneity Test as implemented in PAUP (using an heuristic search with 100 replicates). The analysis of range changes and species turnover (see below) requires the use of a completely resolved tree. When several most parsimonious trees were obtained, tree searches were continued after successive reweighting of characters according to the rescaled consistency index.

Analysis of range changes

Distributional data for the genus *Deronectes* were obtained from Fery & Brancucci (1997) and Fery & Hosseinie (1998); and for *Ilybius* from Larson (1987) for the Nearctic and Franciscolo (1979), Guignot (1933), Nilsson & Holmen (1995) and Nilsson (http://www.bmg.umu.se/BigInst/www/personal/hemsidor/an_big/an_ny.htm) for the Palaearctic. For the analysis of geographical ranges locality information as presented in these papers was converted into shaded maps. The degree of sympatry is defined as the percentage of the more restricted clade's range overlapped by its more

Table 1 List of the species used in the study, with the general distribution, locality of the studied specimen, name of the collector and Genbank accession numbers for the sequences obtained

Species	Geographical range	Sample locality	Collector	16S	COI
Genus *Deronectes* Sharp 1882					
D. abnormicollis Semenow 1900	C Asia	Chimkent Region (Kazakhstan)	H.Fery	AF309250	AF309307
D. algibensis Fery & Fresneda 1988	S Spain	Cadiz (Spain)	I.Ribera	AF309261	AF309318
D. angelinii Fery & Brancucci 1997	Italy	San Ponzo (Italy)	I.Ribera	AF309268	AF309325
D. angusi (Fery & Brancucci 1990)	C Spain	Lugo (Spain)	I.Ribera	AF309253	AF309310
D. aubei (Mulsant 1843)	N Spain, S France, N Italy	Moulinet (France)	I.Ribera	AF309269	AF309326
D. bicostatus (Schaum 1864)	C & NW Iberia	Portalegre (Portugal)	I.Ribera	AF309263	AF309320
D. costipennis Brancucci 1983	C & NW Iberia	Leon (Spain)	D.Bilton	—	AF309324
D. delarouzei (Jacquelin du Val 1857)	N Spain, S France	Lleida (Spain)	P.Aguilera	AF309254	AF309311
D. depressicollis (Rosenhauer 1856)	SE Spain	Granada (Spain)	I.Ribera	AF309264	AF309321
D. fairmairei (Leprieur 1876)	SW Mediterranean	Gourrama (Morocco)	I.Ribera	AF309255	AF309312
D. ferrugineus Fery & Brancucci 1987	C & NW Iberia	Leon (Spain)	I.Ribera	AF309265	AF309322
D. fosteri Aguilera & Ribera 1996	NE Spain	Barcelona (Spain)	P.Aguilera	AF309260	AF309317
D. hispanicus (Rosenhauer 1856)	Iberia, S France, N Morocco	Albacete (Spain)	I.Ribera	AF309258	AF309315
D. lareynii (Fairmaire 1858)	Corsica	Corsica	I.Ribera	AF309259	AF309316
D. latus (Stephens 1829)	N & Central Europe	New Forest (England)	I.Ribera	AF309252	AF309309
D. moestus (Fairmaire 1858)	W Mediterranean	Corsica	I.Ribera	AF309256	AF309313
D. opatrinus (Germar 1824)	Iberia, S France	Barcelona (Spain)	P.Aguilera	AF309257	AF309314
D. persicus Peschet 1914	Iran	Iran	H.Fery	AF309251	AF309308
D. platynotus (Germar 1834)	C & E Europe	Krivoklatska (Cesk Republic)	J.Statszny	AF309267	—
D. semirufus (Germar 1845)	Alps, Italy	Monti (France)	I.Ribera	AF309270	AF309327
D. theryi (Peyerimhoff 1925)	Morocco	Tizi-*n*-test (Morocco)	I.Ribera	AF309262	AF309319
D. wewalkai Fery & Fresneda 1988	C Spain	Avila (Spain)	H.Fery	AF309266	AF309323
D. youngi Fery & Hosseinie 1998	SW Iran	Kohkiluyeh (Iran)	H.Fery	AF309249	AF309306
Outgroups					
Laccornis oblongus (Stephens 1835)		Mount Bog (Scotland)	D.Bilton	AF309241	AF309298
Suphrodytes dorsalis (Fabricius 1787)		Norfolk (England)	D.Bilton	AF309242	AF309299
Hydroporus planus (Fabricius 1781)		Burgos (Spain)	I.Ribera	AF309243	AF309300
Oreodytes davisii (Curtis 1831)		Avila (Spain)	H.Fery	AF309244	AF309301
Nebrioporus baeticus (Schaum 1864)		Sevilla (Spain)	I.Ribera	AF309245	AF309302
N. carinatus (Aube 1838)		Leon (Spain)	I.Ribera	AF309246	AF309303
Stictotarsus duodecimpustulatus (Fabricius 1792)		Cadiz (Spain)	I.Ribera	AF309247	AF309304
Scarodytes halensis (Fabricius 1787)		Barcelona (Spain)	H.Fery	AF309248	AF309305

Table 1 *Continued*

Species	Geographical range	Sample locality	Collector	16S	COI
Ilybius subaeneus group *sensu* Nilsson (2000)					
I. aenescens Thomson 1870	W Palaearctic	Finland	T.Berendonk	AF309294	AF309350
I. angustior (Gyllenhal 1808)	Holarctic (Boreal)	Rundvile (Sweden)	A.Nilsson	AF309289	AF309345
I. anjae Nilsson 1999	E Palaearctic	Kuril Islands (Russia)	N.Minakawa	AF309295	AF309351
I. apicalis Sharp 1873	E Palaearctic	Kuril Islands (Russia)	N.Minakawa	AF309279	—
I. ater (De Geer 1774)	W Palaearctic	West Norfolk (UK)	I.Ribera	AF309287	AF309343
I. biguttulus (Germar 1824)	Nearctic	New York (US)	K.Miller	AF309282	AF309338
I. chishimanus Kono 1944	E Palaearctic	Kuril Islands (Russia)	N.Minakawa	AF309288	AF309344
I. crassus Thomson 1856	N Europe (Boreal)	Strycksele (Sweden)	A.Nilsson	AF309297	AF309353
I. discedens Sharp 1882	Nearctic, E Palaearctic	Kuril Islands (Russia)	N.Minakawa	AF309296	AF309352
I. fenestratus (Fabricius 1781)	Palaearctic	Rundvile (Sweden)	A.Nilsson	AF309291	AF309347
I. fraterculus LeConte 1862	Nearctic	Alberta (Canada)	I.Ribera	AF309281	AF309337
I. fuliginosus (Fabricius 1792)	Palaearctic	Norfolk (England)	I.Ribera	AF309293	AF309349
I. guttiger (Gyllenhal 1808)	C & N Europe	Finland	T.Berendonk	AF309285	AF309341
I. meridionalis Aubé 1836	SW Europe	Corsica	I.Ribera	AF309292	AF309348
I. quadriguttatus (Lacordaire 1835)	Europe	Eping Forest (UK)	I.Ribera	AF309283	AF309339
I. quadrimaculatus Aube 1838	W Nearctic	British Columbia (US)	I.Ribera	AF309290	AF309346
I. similis Thomson 1856	N Palaearctic	Västerbotten (Sweden)	J.Bergsten	AF309284	AF309340
I. subaeneus Erichson 1837	Holarctic	Rundvile (Sweden)	A.Nilsson	AF309286	AF309342
I. vittiger (Gyllenhal 1827)	N Holarctic	Västerbotten (Sweden)	A.Nilsson	AF309280	AF309336
Outgroups					
Ilybiosoma lugens (LeConte 1852)		California (US)	A.Cognato	AF309271	AF309328
I. seriatum (Say 1823)		New York (US)	K.Miller	AF309272	AF309329
Platambus maculatus (Linnaeus 1758)		Burgos (Spain)	I.Ribera	AF309273	AF309330
Agabus didymus (Olivier 1795)		Burgos (Spain)	I.Ribera	AF309274	AF309331
Agabus bipustulatus (Linnaeus 1767)		Albacete (Spain)	I.Ribera	AF309275	AF309332
Ilybius subtilis (Erichson 1837)		Västerbotten (Sweden)	A.Nilsson	AF309276	AF309333
I. albarracinensis (Fery 1986)		Sa. Estrela (Portugal)	I.Ribera	AF309277	AF309334
I. chalconatus (Panzer 1796)		Anti-Atlas (Morocco)	P.Aguilera	AF309278	AF309335

widespread sister (Chesser & Zink 1994; Barraclough & Vogler 2000), and was calculated for sister clades at all nodes in the phylogenies.

Sympatry and overlap of geographical areas were plotted against a measure of node age according to Barraclough & Vogler (2000). Node age reflects the relative time since speciation and was estimated from molecular branch lengths on the most parsimonious trees, calculating the branch lengths from the tips to each node. Branch lengths were fitted using maximum likelihood (ML) as implemented in PAUP. Starting with a one-parameter model of sequence evolution, we used log likelihood ratio tests to test for significant improvements of adding parameters to the model (Goldman 1993). To estimate node ages we fitted ML branch lengths assuming a molecular clock and compared the likelihood to that obtained assuming no clock (Felsenstein 1981). Where the unconstrained branch lengths differed significantly from those fitted assuming a molecular clock, branch lengths were scaled according to the method of non-parametric rate smoothing (NPRS) of Sanderson (1997), as implemented in TREEEDIT v1.0a4-61 (Andrew Rambaut and Mike Charleston, http://evolve.zoo.ox.ac.uk/). This approach does not assume a molecular clock, but assumes that rates of change tend to be similar between adjacent branches on the tree. It produces an ultrametric tree by minimizing the sum of squared changes in rate between ancestor and descendant branches across the tree.

Comparison of species turnover

To compare patterns of species turnover between *Deronectes* and *Ilybius* we used the log-lineage through time approach of Nee and colleagues. For each clade, we plotted the logarithm of the cumulative number of lineages against the molecular distance of each node from the root. Under a constant birth–death model, we expected a straight line of slope $b - d$ (where b = speciation rate and d = extinction rate), but with an upturn in the number of lineages towards the present with slope b (Harvey *et al*. 1994; Nee *et al*. 1994, 1995). Levels of turnover between the two clades can be distinguished by the absolute values of b and d, which will be higher in the clade with the highest species turnover (even if the difference $b - d$ has the same value in both). However, in the present example, sampling will also affect the shape of plots, since we included only 43% and 58% of *Deronectes* and *Ilybius* species, respectively. Incomplete sampling is expected to lead to an apparent decrease in diversification rate over time, because recently formed lineages tend to be underrepresented in the sample.

To test for significant differences in species turnover rates between the clades, we therefore use a modified version of the Monte-Carlo constant rates method introduced by Pybus & Harvey (2000; see also Zink & Slowinski 1995). The method calculates a summary statistic, γ, of the relative locations of internal nodes within a phylogeny (details in Pybus & Harvey 2000). For a constant speciation rate model, and phylogenies derived from a complete sample of species, the statistic has a standard normal distribution. Significant departure towards negative values means that the internal nodes are relatively too close to the root, whereas positive values mean that nodes are relatively too close to the tips, compared to the constant speciation rate model. To assess the expected distribution of γ for our study, we performed Monte-Carlo simulations. For *Deronectes*, we simulated a phylogenetic tree of 53 species (the total number of known species in the genus) under a constant birth rate model, then removed 30 species at random (i.e. we kept only 23 species, the same number of species we included in our analysis), retaining the topology and node ages for the remaining species. Similarly, we simulated a phylogeny of 33 species for *Ilybius*, then removed 15 species. For each we calculated γ, and the difference between the values of γ obtained for *Deronectes* and for *Ilybius*. We repeated this procedure 1000 times. If *Deronectes* has experienced greater species turnover than *Ilybius* we would expect it to have a greater proportion of recent nodes, i.e. display significantly greater value of γ, than *Ilybius*. Hence, the one-tailed probability under the null model is the proportion of trials in which the simulated difference in γ between the two clades is greater than or equal to the observed difference. Simulations were programmed in VISUAL BASIC in EXCEL.

Results

Phylogenetic analysis and molecular evolution

Polymerase chain reaction (PCR) amplification was successful for 22 specimens of *Deronectes* and eight outgroups, with a single failure for each of the COI and 16S data set (see Table 1). Length variation in the amplified fragment of 16S rRNA gene was minimal, ranging from 506–509 bp in the ingroup, to 512 bp in the outgroup, whereas COI did not exhibit length variation. Uncorrected '*p*' distances within the ingroup varied between 0.2% and 8.9% (maximum 12.8% in the outgroup) in 16S, and were about twofold higher in COI (Table 2). Parsimony analysis produced a small number of trees from either data partition, with fewer steps and higher consistency in the 16S partition compared to COI (Table 2). Trees obtained from either partition were similar with respect to a set of critical nodes (Table 3), and the signals in each partition were not significantly in conflict with each other according to the ILD test of Farris *et al*. (1994) ($P = 0.72$). The combined dataset produced three most parsimonious trees of 1632 steps and CI = 0.393, with four nodes unresolved in the strict consensus (Fig. 1). One of these trees was identical to

Table 2 Length of the aligned sequences of 16S rRNA and COI for *Deronectes* and *Ilybius*, with character variability and tree statistics

Partition	Size	Inf	p distance	Length	No. trees	CI
Deronectes						
16S	518	125	0.2–8.9 (12.8)	419	9	0.499
COI	802	253	0.4–16.4 (17.5)	1199	65	0.361
16S + COI				1632	3	0.393
Ilybius						
16S	516	102	0.0–9.4 (11.5)	369	15	0.507
COI	723	227	0.97–15.8 (17.4)	1054	6	0.386
16S + COI				1440	3	0.412

Size, size of the (aligned) data matrix; inf, parsimony informative characters; p distance, range of uncorrected distances within the ingroup and (in brackets) maximum distance with outgroups; length, length of the most parsimonious trees; No. trees, number of most parsimonious trees; CI, consistency index.

the single tree obtained after one round of successive weighting and this tree was selected for all subsequent analyses.

Noteworthy results are the monophyly of the genus *Deronectes* (node 1); the monophyly of the eastern Mediterranean *parvicollis* plus the western *latus* groups (node 2), and their sister relationship to the remainder of the western groups; the sister relationship of the *aubei* group with *D. platynotus* (node 6); the sister relationship of *D. theryi and* the *fairmairei and moestus* groups (node 9), and the polyphyly of the *opatrinus* group (nodes 5 and 8). Most of these nodes have good support and are also recovered in the separate analysis of either data partitions (Table 3).

PCR amplification was successful for 19 species of *Ilybius* and eight outgroups, with a single failure in the COI data (Table 1). These sequences revealed features and tree statistics generally similar to the *Deronectes* data set, but with slightly higher pairwise distances throughout the group and trees of slightly higher CIs obtained in separate and combined phylogenetic analysis (Table 2). The 16S and COI data partitions were not significantly incongruent ($P = 0.11$) and the simultaneous analysis produced three most parsimonious trees of 1440 steps and CI = 0.412 (Fig. 2). None of these trees was identical to the single tree from successive weighting but one of them differed only at a single node and therefore was chosen for the subsequent analyses. The recovery of significant nodes in the separate and combined analyses is summarized in Table 3.

This tree shows the monophyly of the genus *Ilybius* as redefined by Nilsson (2000) (node 1); the clade formed by *I. vittiger*, *I. discedens*, *I. angustior* and *I. anjae* (node 3); the sister relationship between *I. biguttulus* and *I. quadrimaculatus* (node 7); and a large clade including most of the exclusively Palaearctic species plus *I. biguttulus* and *I. quadrimaculatus* (node 9). These nodes were supported in all analyses and have high Bremer and Bootstrap values (Fig. 2; Table 3). In the consensus tree the position of the species *I. apicalis* (for which there was no COI sequence) was not resolved, and in the reweighted tree it was included among the outgroups.

Table 3 Test of relevant nodes in phylogenetic analysis for *Deronectes* and *Ilybius*

	16S		COI		SA	
node	cons.	rew.	cons.	rew.	cons.	rew. (final)
Deronectes						
1	U	M	M	M	M	M
2	M	M	M	M	M	M
3	N	N	M	M	M	M
4	M	M	M	M	M	M
5	U	N	U	N	M	M
6	M	M	NA	NA	M	M
7	M	M	M	M	M	M
8	U	N	U	N	U	M
9	M	P	M	M	M	M
Ilybius						
1	M	M	M	M	M	M
2	M	M	M	M	U	M
3	M	M	M	M	M	M
4	M	M	U	P	M	M
5	M	M	U	P	M	M
6	U	M	P	N	M	M
7	M	M	M	M	M	M
8	U	N	M	M	M	M
9	M	M	M	M	M	M

Trees were reconstructed as described in the Materials and Methods, based on separate analysis of 16S rRNA, COI and a simultaneous analysis (SA). Results of the consensus (cons.) and the reweighted (rew.) trees are presented. See Figs 1 and 2 for the nodes. M, monophyletic; P, paraphyletic; U, unresolved but consistent with monophyly; N, polyphyletic; NA, not applicable (one of the species missing).

Because of the uncertainty of the position of this species relative to other basal groups (node 2) this species was excluded from the analysis of range changes which is conducted on ingroup taxa only.

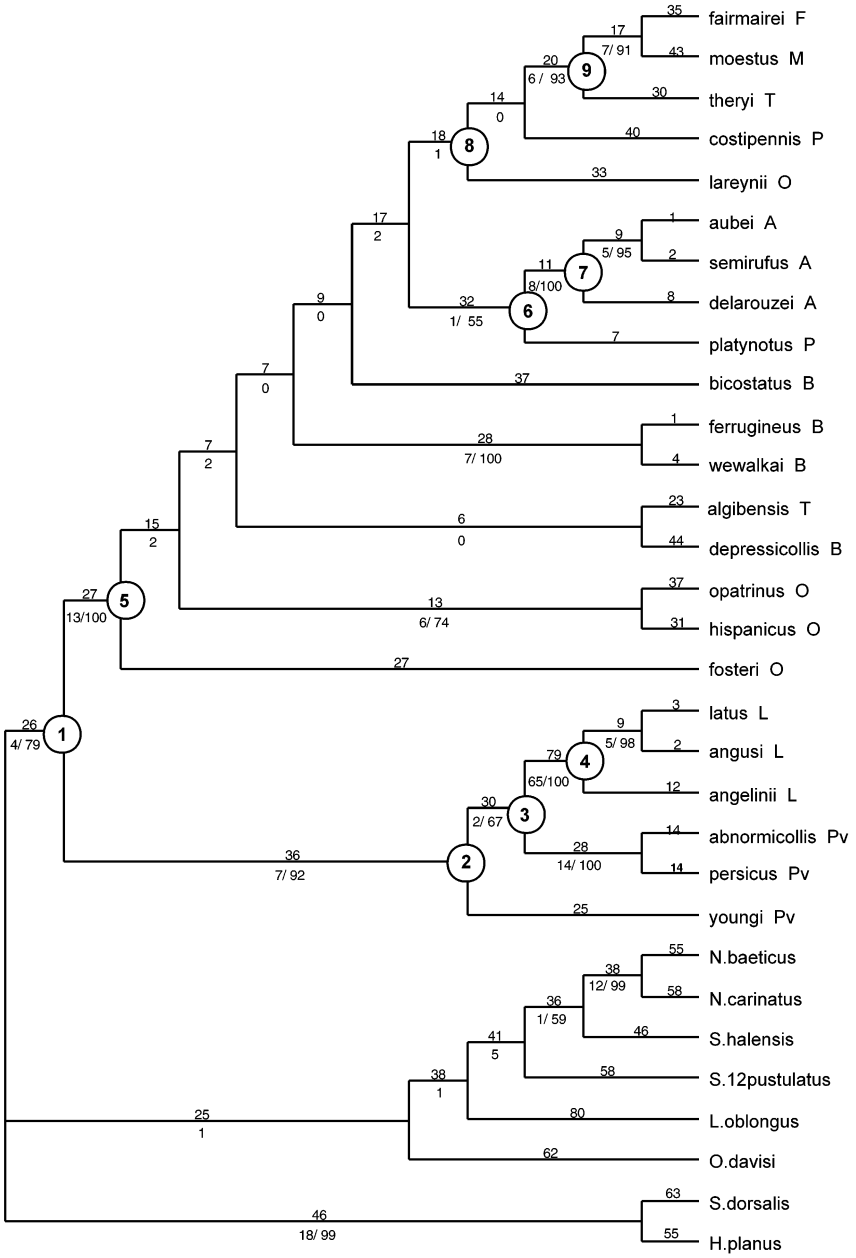

Fig. 1 Phylogenetic hypothesis for *Deronectes* based on a simultaneous analysis of 16S rRNA and COI genes. Numbers above the branches are parsimony branch lengths. Numbers below the branches are Bremer Support values and bootstrap proportions (only if > 50%). Nodes not recovered in the strict consensus of three shortest trees are indicated by zero Bremer support. Nodes of particular interest discussed in the text and Table 3 are numbered (encircled). Capital letters after the taxon names refer to the species groups as defined by Fery & Brancucci (1997): O, *opatrinus*; P, *platynotus*; A, *aubei*; B, *bicostatus*; F, *fairmairei*; M, *moestus*; L, *latus*; Pv, *parvicollis*; and T, *theryi*.

Analysis of range changes

For the analysis of sympatry according to Barraclough & Vogler (2000), node age was calculated from ML branch lengths. Estimated parameter values and logL are given in Table 4. Using a two-parameter model and assuming rate variation across sites proved to be significantly better than simpler models (likelihood ratio test $P << 0.001$). We did not consider more complicated models, as further description of the substitution process is unlikely to affect the conclusions we present here. Furthermore, we tested for departure from a molecular clock model. This test was performed on the ingroup only and revealed highly significant deviation from clock-like variation in both *Deronectes* (–logL 5982.0 vs. 6045.4 without and with clock enforced, χ^2 126.7 with 21 degrees of freedom, $P << 0.001$) and *Ilybius* (–logL 5583.7 vs. 5612.5, χ^2 57.6 with 16 degrees of freedom, $P << 0.001$). Estimates of branch lengths (based on the topology of the parsimony trees) were therefore scaled by nonparametric rate smoothing (NPRS). The resulting ultrametric trees with their ML branch lengths based on these calculations are shown in Fig. 3.

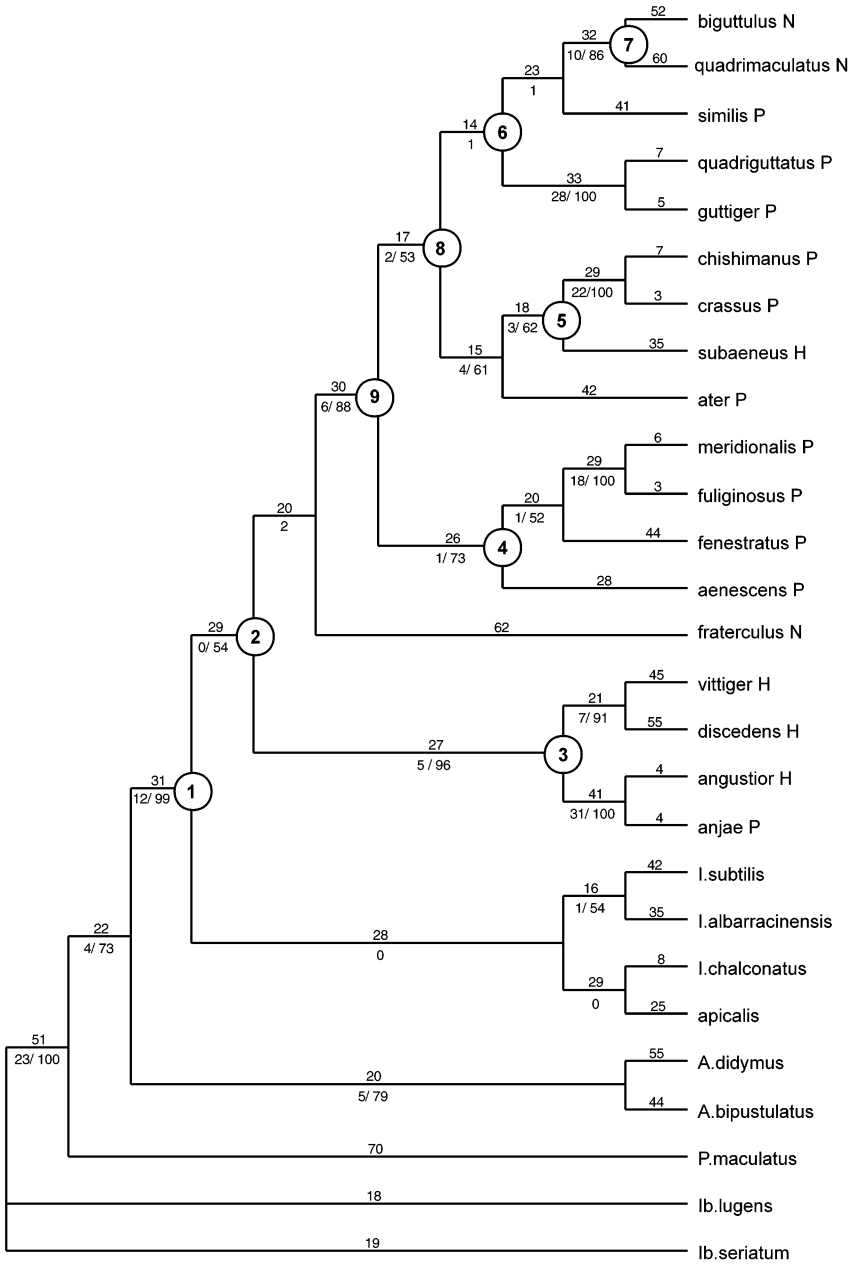

Fig. 2 Phylogenetic hypothesis for *Ilybius* based on a simultaneous analysis of 16S rRNA and COI genes. All annotations as in Fig. 1. The capital letters following the taxon names refer to the general distribution of the species (H, Holarctic; P, Palaearctic; N, Nearctic).

Sympatry vs. node age plots showed a wide scatter of data points, in particular in *Deronectes* (Fig. 4). Comparisons of plots were made by comparing intercepts of the *y*-axis based on linear regression of arcsine transformed sympatry values (Barraclough & Vogler 2000). The fitted intercept in *Deronectes* was 0.20, while that of *Ilybius* was 0.26, suggesting a higher degree of allopatry for the recent nodes of *Deronectes*. However, the confidence intervals for the estimation overlap (minimum and maximum 95% confidence intervals for *Deronectes* and *Ilybius* 0.004–0.587 and 0.013–0.67, respectively) reflected the large scatter of the data points in both genera. In *Deronectes* the number of nodes with zero sympatry (six out of a total of 22) was higher than in *Ilybius* (three out of 17), two of which involved less completely sampled Nearctic taxa. Most of the zero-sympatry nodes of *Deronectes* involved species pairs in the west-Mediterranean, for which the missing species were not expected to overlap with their potential sisters (as identified by their morphology, Fery & Brancucci 1997). The inclusion of these missing species was expected to increase the number of zero-sympatry nodes in *Deronectes* but not *Ilybius*.

Table 4 Parameters of the Maximum Likelihood models for the phylogenies of *Deronectes* and *Ilybius* used in the analysis (see Figs 1,2,3)

ML model	Transition/transversion ratio	γ shape parameter	– LogL	χ^2	*P*
Deronectes					
1 parameter	NA	NA	10458.6		
2 parameter	1.1	NA	10273.5	370	<< 0.001
2 parameters plus rate variation across sites	1.1	0.16	8932.2	2683	<< 0.001
Ilybius					
1 parameter	NA	NA	9344.4		
2 parameter	1.4	NA	9112.5	464	<< 0.001
2 parameter plus rate variation across sites	2.1	0.16	7900.7	1212	<< 0.001

Significant differences between increasingly complex models were tested by comparing Maximum Likelihood ratios (2 × difference in LogL has a χ^2 distribution with 1 degree of freedom). Values of the χ^2 refer to the comparison between each model and the preceding model (NA, not applicable).

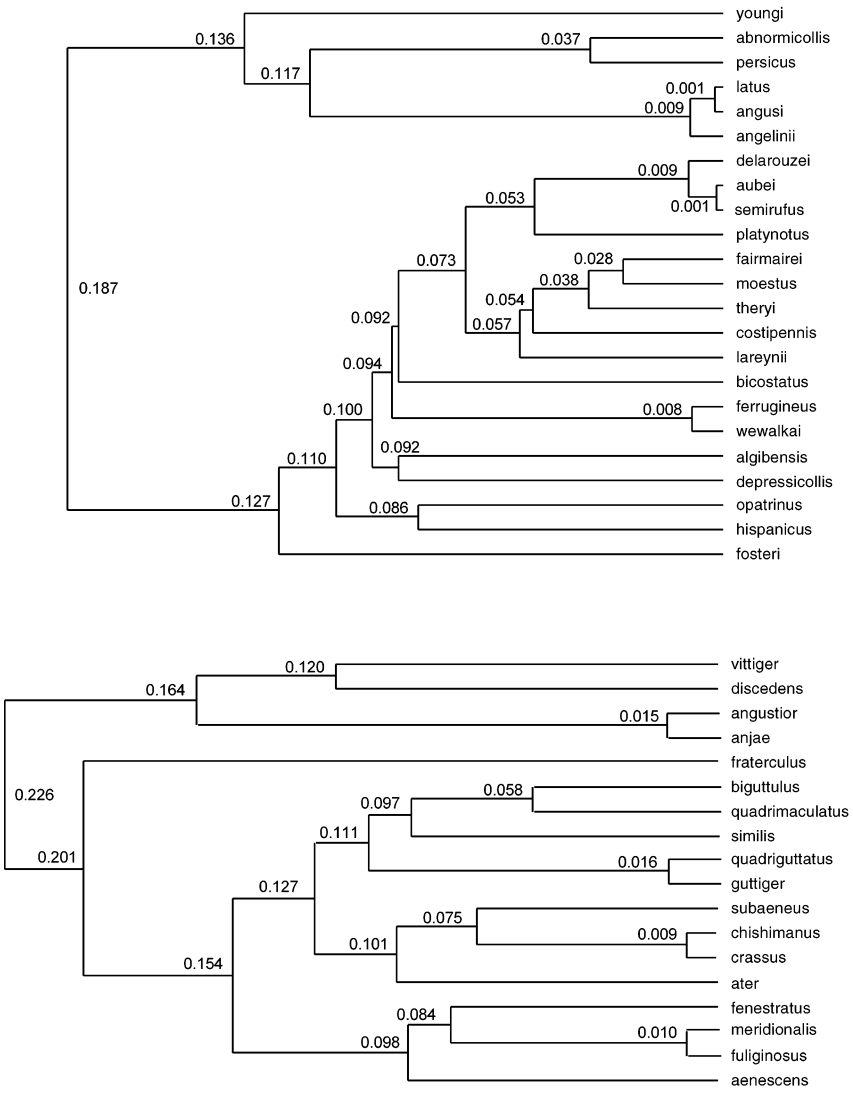

Fig. 3 Ultrametric trees for the genera *Deronectes* (top) and *Ilybius* (bottom), obtained with Maximum Likelihood (two parameters and rate variation model) and nonparametric rate smoothing (see Materials and methods and Table 4). Numbers in nodes are their estimated relative age used in estimations of range changes and lineage-though-time plots in Figs 4 and 5.

Of the five nodes with the highest sympatry in *Deronectes*, four involved the two widespread species *D. moestus* and *D. fairmairei* (the fifth node corresponded to *D. aubei* and *D. delarouzei*, two close relatives which almost overlap entirely). The fact that these two widespread sister species were nested within a group of largely allopatric species strongly affects the calculation of the degree of sympatry at all deeper nodes, even though most of the remaining species had fully allopatric ranges.

Comparison of species turnover

We visualized the dynamics of cladogenesis in each group, using lineage-through-time plots according to Nee *et al*. (1994) (Fig. 5). Plots for both lineages were very similar, revealing a levelling off towards the present as expected for incomplete samples. By comparing the observed γ statistics to those obtained by Monte-Carlo simulation for equivalent sampling of species, we cannot reject the constant speciation rate model in either case (*Ilybius* $\gamma = -1.27$, $P = 0.24$; *Deronectes* $\gamma = -0.85$, $P = 0.72$). *Deronectes* displayed a greater frequency of recent splits than *Ilybius*, shown by its more positive γ statistic, but the difference between these two values was not significantly greater than that expected by Monte-Carlo simulation (the simulated difference exceeded the observed difference of 0.42 in 24% of trials, therefore $P = 0.24$). From the present data we found little evidence for differences in the dynamics of cladogenesis between the two groups.

Discussion

Taxonomic conclusions

The genus *Deronectes* includes radiations mainly confined to either western or eastern Mediterranean regions, but in the mtDNA analysis the western and eastern groups were not monophyletic. The western *latus* group was contained within the eastern *parvicollis* group which is paraphyletic due to the basal position of *D. youngi* (a taxon placed in a separate subsection of the *parvicollis* group). The affinity of both groups is not unexpected, as Fery & Hosseinie (1998) recognized similarities in their aedeagus structure. It can be expected that the other major eastern lineage, the *doriae* group, which was the only species group not included in our analysis, will be close to other western groups, possibly *D. opatrinus* and *D. hispanicus* (Wewalka 1970). Among the species groups recognized by the traditional taxonomy, the *bicostatus* and *opatrinus* groups are found to be paraphyletic, and the former has possibly been defined on plesiomorphic characters (e.g. the presence of costae in the elytra). In agreement with their placement in our analysis, morphological similarities of members of the *bicostatus* group with the *theryi* group were also noted by Fery & Brancucci (1997).

In the genus *Ilybius*, the only available information on the phylogeny of Palaearctic species is a preliminary proposal for the Nearctic species by Larson (1987) and an unpublished thesis by Berglund (1998). The mtDNA study supports the monophyly of the genus *Ilybius* as redefined by Nilsson (2000), including species formerly assigned to the genus *Agabus* (*chalconatus*, *erichsoni* and *opacus* groups, here represented by *I. albarracinensis*, *I. chalconatus* and *I. subtilis*), and confirmed these as the sister of the *Ilybius subaenus* (= *Ilybius* in the traditional sense) group. The monophyly of the *I. subaeneus* group is, however, not clear due to the uncertainty in the position of *I. apicalis*, in part because sequence information for this taxon is incomplete. Berglund (1998) found this species to be basal to the *I. subaeneus* group, similar to its position in one of the three shortest trees in our parsimony analysis. Within the *I. subaeneus* group, several species groups were in agreement with the findings of Berglund (1998), i.e. those groups defined by node 4 in Fig. 2 (although with the inclusion of *I. quadrimaculatus* in Berglund 1998), node 5 and the basal position of *I. discedens*, *I. vittiger* and *I. fraterculus*. In agreement with Berglund (1998), the ancestral distribution seems to be Holarctic, with a vicariant split between *I. fraterculus* (Nearctic) and the remaining species (Palaearctic, with the exclusion of the secondarily Nearctic species in node 7) (Fig. 2).

Evolutionary dynamics of lotic vs. lentic lineages

The differences in temporal dynamics of lentic and lotic habitats is likely to require different strategies for long-term population persistence. As a consequence, lentic species could be expected to be dispersive whereas lotic species may persist without long-range movements (Ribera & Vogler 2000). Consistent with this, the lentic *Ilybius* has very large, frequently continent-wide, distributional ranges while the lotic *Deronectes* has generally smaller ranges, with some species limited to a single mountain range. The reduced ranges (and presumed small population sizes) would result in the expectation of higher species turnover (speciation and extinction rates) in lotic lineages due to their presumably higher genetic structure and thus greater propensity for allopatric speciation but reduced population persistence.

Our findings provide little evidence in support of these expectations. *Deronectes* displays a higher frequency of relatively recent nodes than *Ilybius*, consistent with higher species turnover, but the difference is not significant compared to expected differences under a constant speciation rate model. Although we cannot rule out that differences would be found if more species were sampled, with current sampling levels the two clades display very similar patterns. In addition, the two clades displayed similar patterns of range overlap in sympatry plots, consistent with range movements occurring in both groups. *Deronectes* exhibits a

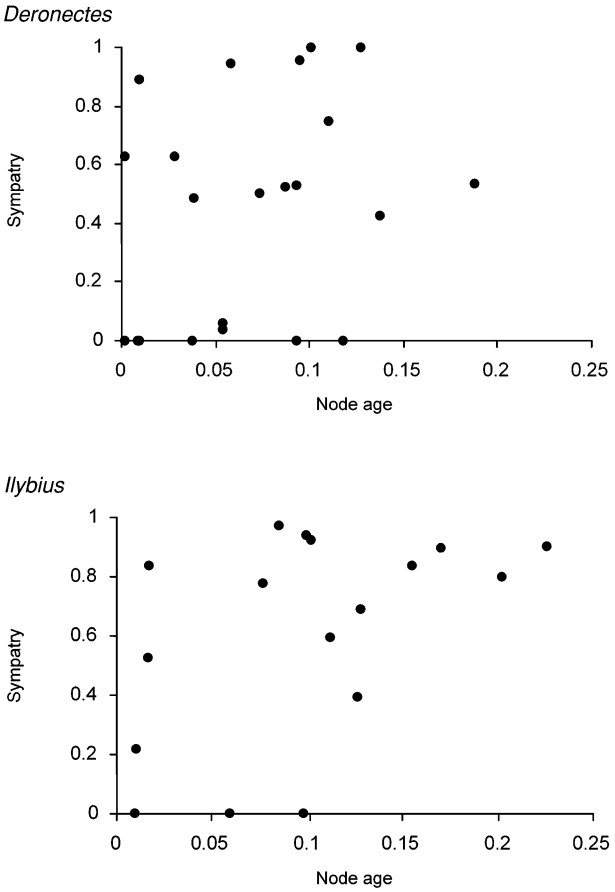

Fig. 4 Plots of the degree of sympatry against relative node age in *Deronectes* (top) and *Ilybius* (bottom). The *y*-axis is the degree of sympatry. The *x*-axis represents the relative node age, with units representing a measure of branch length from the tip of the tree to the focal node, under the assumptions of a molecular clock and scaled with the NPRS method, as given in Fig. 3.

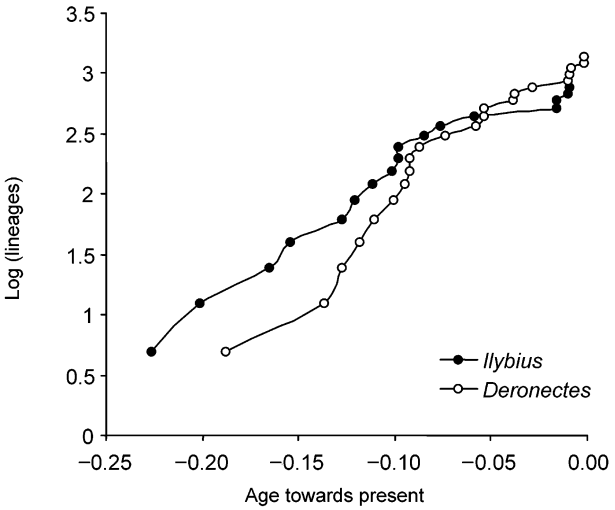

Fig. 5 Lineages-through-time plots for *Deronectes* and *Ilybius*. The *y*-axis is the log number of lineages. The *x*-axis represents the relative age towards present, based on NPRS-scaled branch lengths given in Fig. 3. Both plots are represented together for comparison, although this implies equivalent rates of molecular variation in both genera, an assumption necessary for a direct comparison of relative rates of species turnover.

higher proportion of recent nodes with allopatric distributions, again consistent with the hypothesis, but overall the patterns were statistically indistinguishable using current methods. The results are surprising given the major difference in habitat affinity and range size between the two groups. There are two potential explanations: current methods and/or sampling levels are not sufficient to detect differences that exist between the clades, or there really is no difference between them.

Methodological issues

A number of potential limitations affect our analyses, perhaps explaining our failure to detect significant differences between the two clades. First, the assessment of the degree of range movements from plots of sympatry against node age is potentially a useful tool for establishing the geography of speciation and levels of range movements but the method is new and its performance has been little explored. The significance of the comparisons was assessed by comparing confidence intervals of intercepts fitted by linear regression (Barraclough & Vogler 2000). However, inferences about the general trends of geographical movements from these plots may be sensitive to the behaviour of single species with deviating properties. If a single species has experienced a rapid expansion to its range, all nodes below that species may have a sympatry index close to unity even if other species within the clade are fully allopatric. The analysis of *Deronectes* could be affected by this phenomenon, for example in the case of *D. fairmairei*, a species with a comparatively large range in the Mediterranean. Additional methods could be used to identify such cases. Statistical methods could be used to identify heterogeneity of pattern within study clades. Alternatively, recent range shifts could be recognized by low intraspecific genetic variability, which should be reduced compared to species with older ranges (Hewitt 1996; Avise 2000). Preliminary data for *D. fairmairei* suggest a high degree of genetic homogeneity compatible with the hypothesis of a recent range expansion (four specimens studied from Spain and Morocco had identical sequences, unpublished results), and perhaps masking otherwise low range movements within the clade.

A further, more general, problem of making comparisons between lineages based on species-level phylogenies is

the issue of sampling and comparability of terminal taxa ('species'). The units of our phylogenies are species morphologically defined by taxonomists in the most recent revisions. The operational species concept is thus that of morphological diagnosability, which may be highly idiosyncratic and hardly comparable between or even within a genus or species group. For example, some very closely related species of *Deronectes* have clear diagnostic characters in the male aedeagus, colour of the body, or male secondary sexual characters (e.g. the *D. latus* group, node 4 in Fig. 1, the *D. aubei* group, node 7, or the species pairs *D. ferrugineus*/*D. wewalkai*; Fery & Brancucci 1997). Genetic differences among these species are rather shallow (uncorrected 'p' distances between *D. ferrugineus* and *D. wewalkai* 0.004, *D. latus* and *D. angusi* 0.004, *D. latus* and *D. angelini* 0.018; and *D. aubei* and *D. semirufus* 0.002). All of these species are allopatric with the only exception of *D. aubei* and *D. semirufus*, with a sympatry index of 0.63. On the contrary, *D. moestus*, with one of the largest ranges and overlapping with most of the remaining western Mediterranean species of the genus, has a rather simple male aedeagus and no conspicuous characters suitable for the diagnosis of species. Preliminary results indicate that genetic differences between populations of *D. moestus* from the north of its distributional area (Corsica, north Italy and northeast Spain) and those from the southwest (south Spain and Morocco) are comparable to differences between other sister species pairs, ranging between 0.003 and 0.026 (six specimens studied, unpublished results). This raises the possibility of *D. moestus* being a complex of unrecognized species due to the lack of diagnostic morphological characters to separate populations, thus artificially decreasing the number of recent splits in its lineage.

A related problem is the possible existence of undiscovered species. Species of *Ilybius* have northern distributions in the entomologically best known areas, are relatively large and conspicuous (10–15 mm) and have larger ranges, factors facilitating their discovery and description (Gaston 1991, 1994). Thus, of the 33 recognized species, 26 were described before 1900. Only two have been described in the last 50 years (Nilsson 2000), both from the eastern Palaearctic and both of them closely related to their sisters—the one included in this study, *I. anjae*, is identical in 16S sequence to its sister, *I. angustior*. In contrast, species of *Deronectes* have a more southerly distribution, are smaller in size (3.5–6 mm), and have restricted geographical ranges, factors contributing to a lower probability of being discovered and described. Of the 53 currently recognized species, only 10 were described before 1900, 39 were described in the last 50 years, 17 of them in the most recent revision (Fery & Brancucci 1997; Fery & Husseinie 1998). Some of these newly described species are known only from a few specimens in the type locality, and it is likely that new species will be described in the future. Each of these factors could underestimate the number of allopatric sister taxa and the rate of species turnover in *Deronectes*, but not in *Ilybius*. Therefore, improved data and taxon sampling may still result in strong support for the proposed hypothesis.

The issue of scale

An alternative possibility is that, even after accounting for methodological problems, there really are no differences in species turnover and levels of relative range movement between *Ilybius* and *Deronectes*. This may seem surprising given the major differences in habitat affinity and range size between them. In fact, the macroevolutionary consequences of differences in range size remain contentious, with contrasting hypotheses regarding speciation and extinction probabilities (see Gaston & Chown 1999 for a recent review). On the one hand, large ranges may be associated with higher local densities and higher genetic cohesion, thus decreasing the probability of speciation and extinction (a line of thought that goes back to Darwin, discussed in Maurer 1999). Species with larger ranges should also have lower extinction probabilities (e.g. Jablonski 1986, 1987), contributing to lower overall species turnover. However, a large range size may also increase the opportunity for allopatric speciation (e.g. Rosenzweig 1995), although the shape of this relationship (i.e. if there is an 'optimal' range size maximizing speciation probability, or if the increase is linear) is also not clear (Chown & Gaston 2000).

If no differences in turnover rate are found between *Deronectes* and *Ilybius*, this suggests that similar evolutionary processes have taken place at two very different geographical scales. *Deronectes* and *Ilybius* display very similar patterns of lineages through time even though *Ilybius* occupies more than twice the area inhabited by *Deronectes*. The methods we use take no account of the spatial scale over which diversification proceeds: sympatry plots are based on a relative index (the proportion of overlap among ranges), and lineage-through-time plots are exclusively based on the topology and relative position of the nodes in the phylogenetic tree. However, the differences in gene flow and population structure, as proposed to explain the lotic–lentic differences of total ranges refer to absolute differences in a defined geographical space. New methods are needed to model in detail the effects of spatial scale on speciation and extinction, and to test whether clades occupying very different scales differ in their underlying probabilities of speciation and extinction per unit area. One approach might be to compare assemblages of species within a defined geographical region.

Conclusions

Species level phylogenies provide a means to investigate the timing and spatial dimension of macroevolution within

clades. Comparing related lineages with different life styles and ecology has great potential for elucidating the factors promoting species richness within lineages. However, more work is needed to assess the sensitivity of existing methods to taxonomic and sampling problems, and to investigate the link between population processes and speciation. In particular, new methods are needed to investigate how spatial scale affects the dynamics of species turnover. Few good examples exist where genetic structure has been compared side-by-side for different species across a particular geographical region. The highly structured habitat of aquatic beetles and the fact that radiations are large enough for the detection of statistical patterns make this group into an ideal study system for across-lineage comparisons.

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