

Reproductive isolation in snails of the genus *Albinaria* (Gastropoda: Clausiliidae)

MENNO SCHILTHUIZEN

Systematic Zoology Group, Institute of Evolutionary and Ecological Sciences, University of Leiden, P.O. Box 9516, NL-2300 RA Leiden, The Netherlands

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Parapatric, morphologically characterized forms of the Mediterranean snail genus *Albinaria* have traditionally been regarded as biological species. Recently, this view has been challenged on the basis of small interspecific genetic distances and cross-breeding experiments. In order to test whether traditionally recognized species are reproductively isolated lineages, two cases of syntopy were analysed by means of isozyme electrophoresis. The data suggest that complete reproductive isolation is present. In one of the two cases, ecological differentiation on a small spatial scale was observed.

ADDITIONAL KEY WORDS:—species concepts – syntopy – allozymes – ecological differentiation – Greece – Crete.

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INTRODUCTION

The snails of the genus *Albinaria* can be found on limestone rocks almost everywhere within an area consisting of Greece, Asia Minor, Cyprus and The Lebanon. The genus is notoriously speciose. In the latest revision of the genus, Nordsieck (1977) distinguished 59 species. Since then, various taxonomic and field studies have resulted in the description of another 15 species (Gittenberger, 1979; Gittenberger, Flach & Reitsma, 1988; Schilthuizen & Gittenberger, 1990, 1991; Wiese, 1989a, b; Schultes & Wiese, 1991, 1992; Gittenberger & Menkhorst, 1992). At the same time, certain authors (e.g. Mylonas *et al.*, 1988; Mylonas, 1992) have criticized this taxonomic system, arguing that species status for many nominal species is doubtful. At first glance, these doubts may seem justified: most species are allo- or parapatrically distributed (see e.g. Gittenberger, 1991), allozymically close or indistinguishable (Kemperman & Degenaaars, 1992; Ayoutanti *et al.*, 1988; Schilthuizen, unpublished data) and under laboratory conditions interfertile (Mylonas *et al.*, 1988). Therefore, an

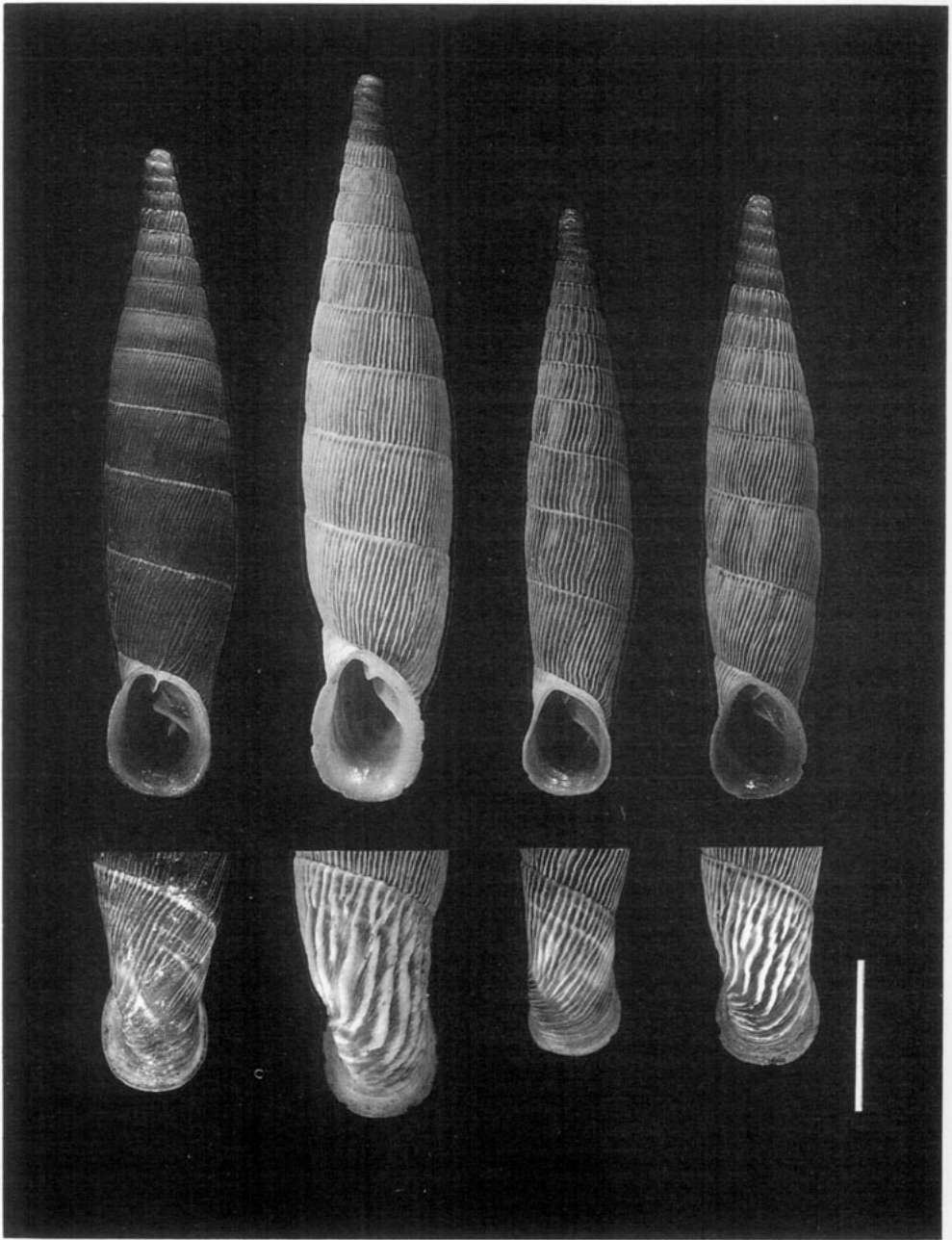


Figure 1. Conchology of the species. Top row: ventral aspect of the shell; bottom row: dorsal aspect of the cervical sculpture. From left to right are shown: *A. ulrikae*, *A. spratti*, *A. hippolyti* and a hybrid of the latter two species. Scale bar = 5 mm.

analogy might be drawn with other cases in malacology where large numbers of nominal taxa proved to be geographical or ecological forms of one or only a few biological species, like in *Cerion* (Woodruff, 1978), *Jaminia* (Matekin, 1959) and *Corbicula* (Woodruff, Kijiviriya & Suchart Upatham, 1993). However, detailed

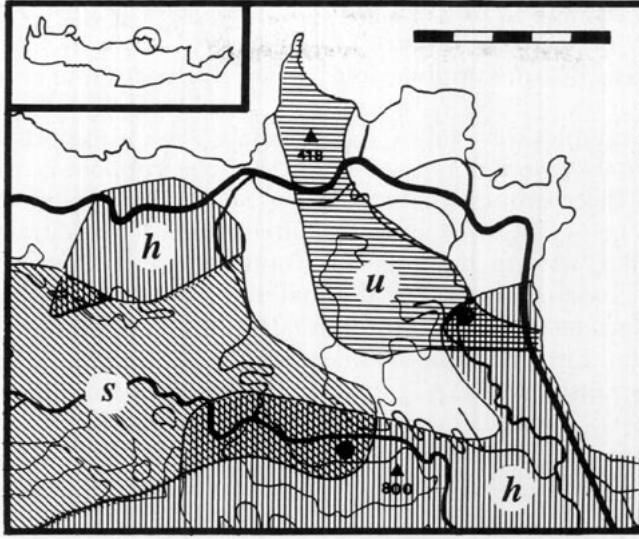


Figure 2. Geographic distribution of *A. hippolyti* (*h*), *A. ulrikae* (*u*) and *A. spratti* (*s*) in a part of Central Crete (based on Schilthuizen & Gittenberger, 1991, Schilthuizen, Welter-Schultes & Wiese, 1993, and on unpublished data). The two study sites are indicated with dots. Scale bar = 5 km.

field studies in *Albinaria* have shown that in those cases where nominal species do occur sympatrically, morphological hybrids are either absent or rare (Schilthuizen, Welter-Schultes & Wiese, 1993; Welter-Schultes, 1992). Similar situations were also reported on by Holyoak (1986) for the related genus *Lampedusa* from Malta.

The present study aims at contributing to the debate by taking a closer look at three nominal *Albinaria* species from Crete, viz. *A. hippolyti* (Boettger, 1878), *A. spratti* (Pfeiffer, 1846) and *A. ulrikae* Schilthuizen & Gittenberger, 1990. Conventional taxonomy would regard these three forms as distinct species on the basis of their morphological distinctness (Fig. 1). However, with regard to their distributions, which are mainly parapatric with only small areas of overlap (Fig. 2), and their allozyme compositions, which show only minute overall differences (Schilthuizen, unpublished data), one might also view them as geographic varieties of a single species. The key to resolving this problem may be reproductive isolation: if the forms do not hybridize in the regions where their ranges overlap, they would represent not only morphologically distinct, but also reproductively isolated lineages, i.e. species in the sense of most accepted species concepts (see Templeton, 1989). If, on the other hand, reproductive isolation is not present, then the alternative view would be more appropriate. In order to test this, two cases of syntopy were examined by means of electrophoresis of codominantly inherited allozymes. This technique has proved to be invaluable in the assessment of reproductive isolation (for other examples concerning molluscs, see Hillis, Rosenfeld & Sanches, 1987 and Backeljau & De Bruyn, 1992).

MATERIAL AND METHODS

Two localities were visited where the species pairs *hippolyti-spratti* and *hippolyti-ulrikae* were known to occur syntopically, i.e. on the same limestone boulders,

within 'cruising distance' of each other. These sites, called HS and HU, respectively, are indicated in Figure 2. At site HU, some habitat segregation could be observed. Before sampling took place, this pattern of segregation was recorded.

At both sites, samples were taken and the snails were transported alive to the laboratory where they were stored at -80°C . For electrophoresis, the snails were homogenized in three drops of homogenizing buffer pH 7.4 and centrifuged at 10 000 g. Starch gels (12% w/v) and two buffer systems (Tris-citrate pH 7.0 and Tris-citrate pH 8.0) were used for the electrophoretic separation of allozymes at 10 V/cm, at 4°C , over 6 hours. For the material from both sites, the following enzymes were detected (nomenclature and IUBNC numbers according to the guidelines of the Fish Genetics Nomenclature Committee [1989]): soluble aspartate aminotransferase (sAAT, 2.6.1.1), major cathodic isozyme of esterase (EST-2, 3.1.1.-), soluble malate dehydrogenase (sMDH, 1.1.1.37) and phosphogluconate dehydrogenase (PGDH, 1.1.1.44). In addition, leucine aminopeptidase (LAP, 3.4.11.1) was detected for the material from site HU and soluble isocitrate dehydrogenase (NADP⁺) (sIDHP, 1.1.1.42) and a minor cathodic isozyme of esterase (EST-1, 3.1.1.-) were detected for the material from site HS. sAAT, LAP and PGDH were stained on Tris-citrate pH 8.0 gels and EST, sMDH and sIDHP were stained on Tris-citrate pH 7.0 gels. Included in the loading of each gel were a few reference samples, consisting of fractions of a homogenate of several hundred individuals of *A. corrugata corrugata*. Mobilities of allozymes were scaled to the mobility of the slowest allozyme in these reference samples.

RESULTS

The spatial distribution of *hippolyti* and *ulrikae* at site HU, a rocky outcrop of 60×35 m, is shown in Figure 3. Although there was a clear segregation of the two species, there was also sufficient overlap to treat this locality as a site of syntopy. At site HS, no such segregation could be observed. Here, *hippolyti* and *spratti* were completely mixed.

Table 1 presents the results of the electrophoretic analysis. At site HU, *EST-2* and *sMDH* were monomorphic. The *PGDH**120 allele showed a much higher frequency in *ulrikae* as compared to *hippolyti*, and the same applied to the *sAAT**100 allele. At *LAP-1* the *106 allele occurred at a high frequency in *hippolyti* but not at all in *ulrikae*. A second locus, *LAP-2*, was monomorphically present in *hippolyti*, but completely absent in *ulrikae* (Fig. 4). In fact, this is the first time this locus was ever observed in *Albinaria*.

At site HS, *sMDH* and *sAAT* were monomorphic. The *PGDH**120 allele occurred at a low frequency in *hippolyti* but was absent in *spratti*. Conversely, the *sIDHP**114 allele was present at an intermediate frequency in *spratti* but was not found in *hippolyti*. Fixed differences were found at the two cathodic esterase loci (Fig. 5): *EST-1* was fixed for the *67 allele in *hippolyti* and for the *100 allele in *spratti*. At *EST-2*, two alleles were found in *hippolyti* (one of which at a low frequency) and two other alleles in *spratti*. One *hippolyti* individual with a vague zymogram, however, may have been homozygous for the commonest *spratti*-allele at this locus (see Fig. 5).

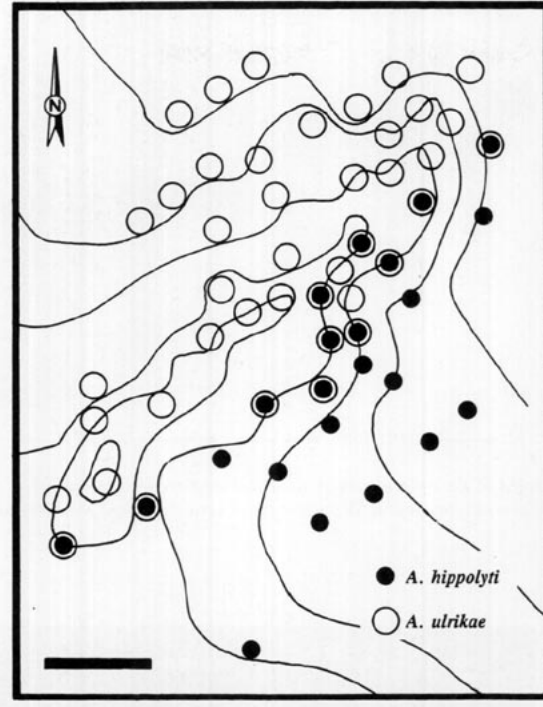


Figure 3. Microgeographic distribution of *A. hippolyti* and *A. ulrikae* at site HU. Scale bar = 10 m. Contours are given at 10 m intervals.

TABLE 1. The frequencies of homo- and heterozygotes in samples from both study sites. For LAP-2 no relative mobilities are given, as this locus was unique to *A. ulrikae* from site HU

Locus	Species			
	site HU: <i>hippolyti</i>	<i>ulrikae</i>	site HS: <i>hippolyti</i>	<i>spratti</i>
<i>sAAT</i>	*130/*130: 14 *100/*130: 1	*130/*130: 4 *100/*130: 6 *100/*100: 6	*130/*130: 19	*130/*130: 22
<i>EST-1</i>	not scored	not scored	67*/67: 15	*100/*100: 14
<i>EST-2</i>	*89/*89: 12	*89/*89: 12	*91/*91: 1 *86/*91: 2 *86/*86: 12	*93/*93: 7 *80/*93: 5 *80/*80: 2
<i>sIDHP</i>	not scored	not scored	*100/*100: 16	*114/*114: 2 *100/*114: 7 *100/*100: 5
<i>LAP-1</i>	*106/*97: 8 *106/*90: 1 *97/*97: 3	*97/*97: 6 *90/*97: 3 *90/*90: 3	not scored	not scored
<i>LAP-2</i>	homozygous: 12	absent	not scored	not scored
<i>sMDH</i>	*127/*127: 14	*127/*127: 16	*127/*127: 19	*127/*127: 22
<i>PGDH</i>	*120/*100: 1 *100/*100: 14	*120/*120: 1 *100/*120: 7 *100/*100: 8	*120/*120: 1 *100/*120: 3 *100/*100: 15	*100/*100: 21

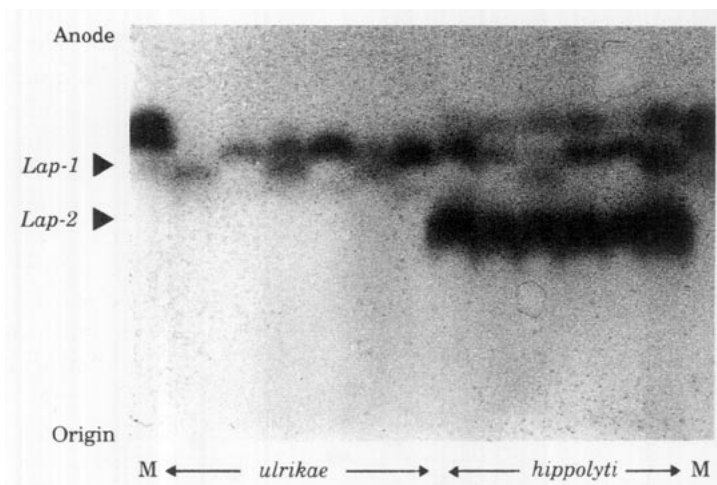


Figure 4. LAP-zymograms of six individuals of *A. ulrikae* and six individuals of *A. hippolyti* from site HU, showing the presence of a second LAP locus in the latter species. Markers are indicated with the letter M.

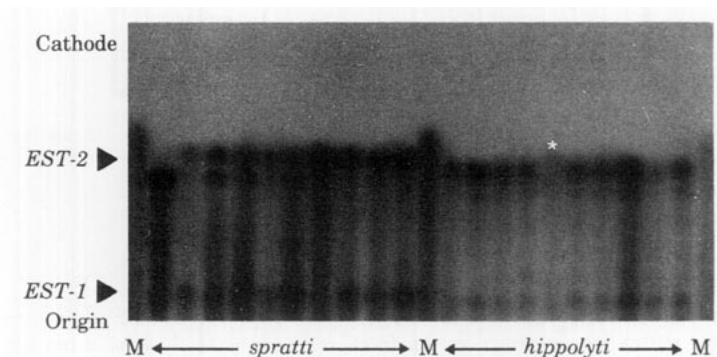


Figure 5. EST-zymograms of ten individuals of *A. spratti* and ten individuals of *A. hippolyti*. An *A. hippolyti* individual with a doubtful zymogram is indicated with an asterisk. Markers are indicated with the letter M.

DISCUSSION

The electrophoretic data show that at both sites, at several loci, one of the species carries alleles that are absent from the other species. Moreover, at site HU, a second LAP locus is expressed in *hippolyti*, while it is absent from *ulrikae*, and at site HS, both species are fixed for alternative alleles at *EST-1*. The data therefore support the hypothesis that these species are reproductively isolated in sympatry. The morphological data point in the same direction, as no intermediate phenotypes were found at site HU, while only one dead shell of possible hybrid origin was found at site HS (Fig. 1).

In the case of *hippolyti* and *ulrikae*, ecological differentiation seems to be present. At site HU, the former species occupies the southeastern flank of the

rock, while the latter is only found on the northwestern flank. In fact, similar types of segregation are found at other sites within the area of sympatry of these two species (unpublished data).

One might argue that, since hybridization apparently does not take place, the distribution pattern described here should not be referred to as parapatric, as this epithet is usually reserved for situations "in which closely related taxa [...] occupy adjacent geographic areas with a very narrow zone of overlap [...] within which hybridization occurs" (White, 1978). On the other hand, the relatively small areas of overlap hardly warrant the use of the term 'sympatric'.

The mainly parapatric distribution of these three species may partly result from historical causes, as Crete has been subdivided into smaller islands several times during its geological history (Anastasakis & Dermitzakis, 1990). Interspecific competition, however, may also play an important role, especially in the case of *hippolyti* and *spratti*, where no ecological differentiation was observed. The ecological differences that exist between *hippolyti* and *ulrikae*, on the other hand, might eventually permit full sympatry of these species.

The three species studied here are morphologically well characterized and, where they meet, reproductively isolated. Even though this may not be true for all geographically replacing forms of *Albinaria*, and the work should be repeated in similar situations, this study demonstrates that inferences about the status of nominal taxa may be incorrect, when drawn from gross distribution and allozymic similarities only.

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