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Parallel evolution of an *sAat*-'hybrizyme' in hybrid zones in *Albinaria hippolyti* (Boettger)

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Populations of the land snail *Albinaria hippolyti* from Crete were sampled across two hybrid zones separating *A. h. aphrodite* from *A. h. holtzi* and *A. h. harmonia* and studied by means of starch gel electrophoresis. At both sites, frequencies of an otherwise rare allele of *sAat* reached 0.70 and 0.18, respectively, in the centres of the hybrid zones. It is argued that the allele is deleterious and that it is maintained in the zones by a balance between elevated mutation rates (at least 1.4×10^{-4} and 3.2×10^{-4}, respectively) and selection (at least 2×10^{-4} and 1.8×10^{-3}, respectively). The observed parallellism may be the result of constraints on the numbers of metabolically active *sAat* variants possible.

Keywords: allozyme electrophoresis, hybrid zones, hybrizymes, mutation, natural selection, snails.

Introduction

It has long been known that individuals in hybrid zones (areas where genetically distinct populations meet and produce hybrid offspring (Barton & Hewitt, 1985)) often carry characteristics that are not found in either parental phenotype. This is particularly obvious in allozyme studies. After Clarke (1968) first found unexpected allozymes in hybrid zones of the land snail *Partula*, many more such instances were reported. Barton & Hewitt (1985), in reviewing the then existing literature on hybrid zones, found that novel or rare allozymes had been detected in 19 of 23 separate studies. Woodruff (1989) extensively discussed the phenomenon, coining the term 'hybrizymes' for such enzyme variants, and speculated on possible mechanisms by which hybrizymes could arise. His hypotheses included intracistronic recombination, gene conversion and relaxed suppression of mutation. At that time, the available evidence could not resolve the question, although intracistronic recombination in heterozygotes seemed to be the favoured hypothesis (D. S. Woodruff, 1989; Murphy *et al.*, 1990). Recently, however, the first DNA sequence for the coding regions of a 'hybrizyme' allele, an *Adh* variant from a *Geomys* hybrid zone, has become available (Bradley *et al.*, 1993). The results from this study do not support the intracistronic recombination hypothesis, as they show that the novel allele was derived from one of the parental alleles by a single point mutation. If simple mutation were proved to be the primary cause for the origin of hybrizymes, then the question shifts to what maintains the hybrizyme alleles at such high frequencies within hybrid zones?

Kemperman & Degenaars (1992) first reported the occurrence of hybrizymes in pulmonate snails of the Mediterranean genus *Albinaria*. In the present study, a curious case of parallel evolution of a hybrizyme in the Cretan *Albinaria hippolyti* (Boettger) is reported and possible causes for its distribution are discussed.

This study is part of a much larger analysis of hybrid zones in *Albinaria hippolyti*: four of the six subspecies that make up this polytypic species are parapatric, interconnected by hybrid zones (Schilthuizen & Lombaerts, 1992; Schilthuizen *et al.*, 1993; see also Fig. 1). These hybrid zones are very narrow, varying in width between a few tens and a few hundreds of metres. They are characterized by multiple, largely coincident and concordant clines in conchological, anatomical and biochemical characters (Schilthuizen, unpublished data). This paper, however, focuses only on the distribution of *sAat* (soluble Aspartate aminotransferase) alleles in two zones. The majority of the descriptive and analytical results of the work will be published elsewhere.

Materials and methods

Two hybrid zones were sampled: one between *A. h. aphrodite* (Boettger) and *A. h. holtzi* Sturany some

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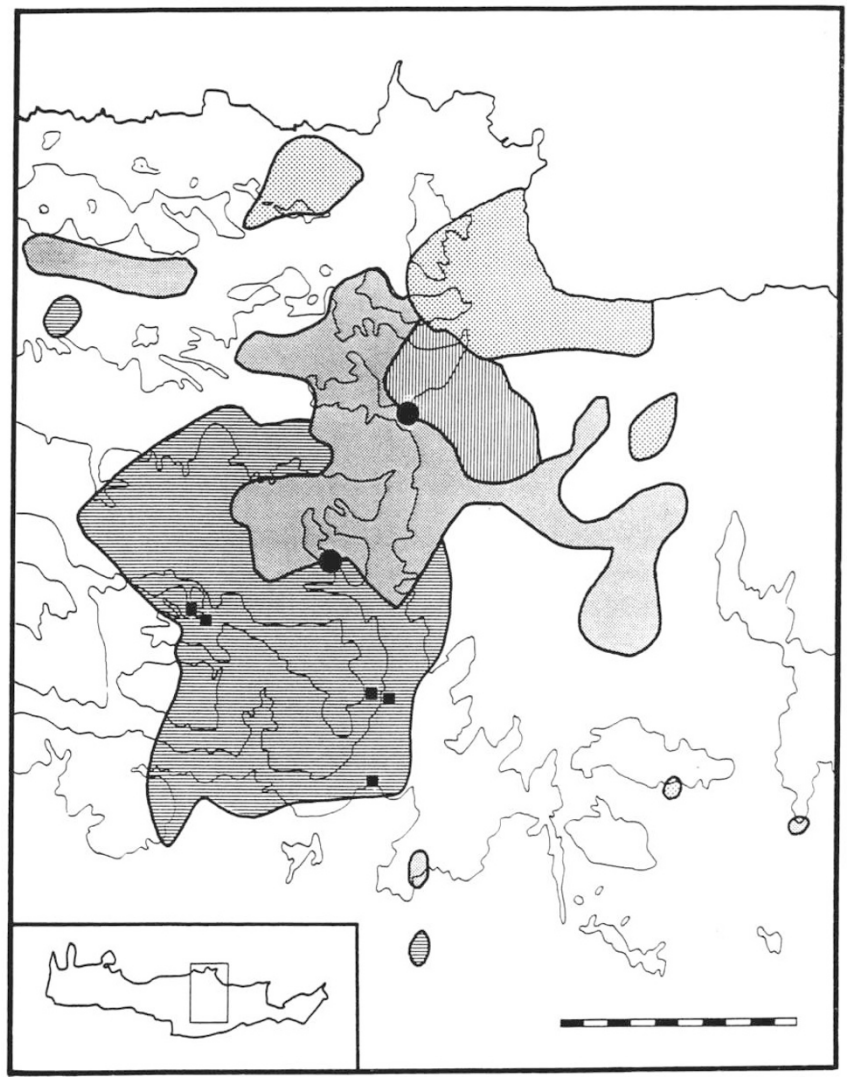

Fig. 1 Area of distribution of *Albinaria hippolyti* in Central Crete. Widely spaced stipples, *A. h. hippolyti*; vertically hatched, *A. h. harmonia*; dense stipples, *A. h. aphrodite*; horizontally hatched, *A. h. holtzi*. The two hybrid zone sites (Kroussónas, between *A. h. aphrodite* and *A. h. holtzi*, and Tílisos, between *A. h. aphrodite* and *A. h. harmonia*) are indicated with dots. Squares indicate five other populations in which the *0.61 allele was found. The scale bar represents 10 km and contours are given at 500 m intervals.

4 km west of the town of Kroussónas, the other between *A. h. aphrodite* and *A. h. harmonia* Schilthuizen *et al.*, some 4 km west of the town of Tílisos (Fig. 1). The former was sampled at 15 sites along a more or less linear transect of some 800 m, and the latter was sampled two-dimensionally at 21 sites in an area measuring approximately 300×700 m. Live snails, predominantly adults, were taken at sites not exceeding 2 m^2 in area. From most sample sites, individuals were killed for electrophoresis. Horizontal starch gel electrophoresis and histochemical staining procedures were employed (as described in Schilthuizen & Lombaerts, 1994) to detect soluble asparate aminotransferase (sAAT, E.C. 2.6.1.1). In all, 118 and 250 individuals were scored from the Kroussónas and the Tílisos zones, respectively. The identity of allozymes was compared across both zones by running homogenates from individuals from both zones alongside each other on the same gels. The allozymes were assigned codes, reflecting their electrophoretic mobilities relative to marker samples consisting of the mixed homogenate of several hundred individuals of *A. corrugata* (Bruguière). Conformance to the Hardy–Weinberg equilibrium was tested for each site where two or more different alleles were found, using the exact probability test provided by BIOSYS-1. As recommended by Rice (1989) and Lessios (1992), the sequential Bonferroni test (Holm, 1979) was used on all 27 tests to determine if any cases showed significant departure from Hardy–Weinberg expectations, with $\alpha = 5\%$.

Results

In each zone, three alleles were detected: *1.31, *1.00 and *0.61 (Fig. 2). In the Tílisos zone, 'pure' *A. h. harmonia* was found to be fixed for the *1.31 allele whereas 'pure' *A. h. aphrodite* was almost fixed for the *1.00 allele. In the Kroussónas zone, both parental taxa

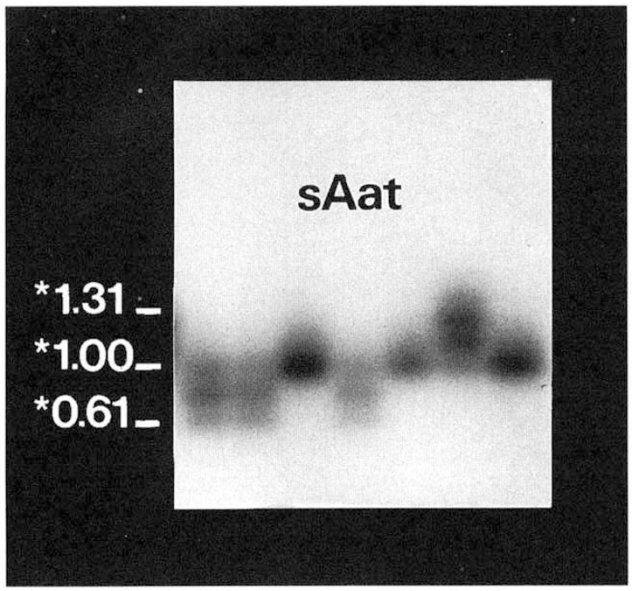

Fig. 2 Starch gel stained for AAT, showing the expression of all three alleles. *sAat**0.61 is the hybrizyme.

were diallelic for *1.31 and *1.00. In both zones, the *0.61 allele was found only in or near the centre of the hybrid zone, reaching very high frequencies (up to 0.70) in the Kroussónas zone and distinctly lower frequencies in the Tílisos zone (up to 0.18). Figures 3 and 4 show the distributions of all three alleles in both zones. Significant departure from the Hardy–Weinberg equilibrium could not be demonstrated in any of the cases.

Discussion

There can be little doubt that the *0.61 allele represents a hybrizyme: in both hybrid zones, its frequency peaks at the centre and tails off to either side. The occurrence of this allele, however, is not restricted to areas of hybridization: it was also found at low to high (0.03–0.86) frequencies at five distant localities in three separate regions within the area of distribution of *A. h. holtzi*; it was not found in any of the approximately 70 other samples of *A. hippolyti* that were

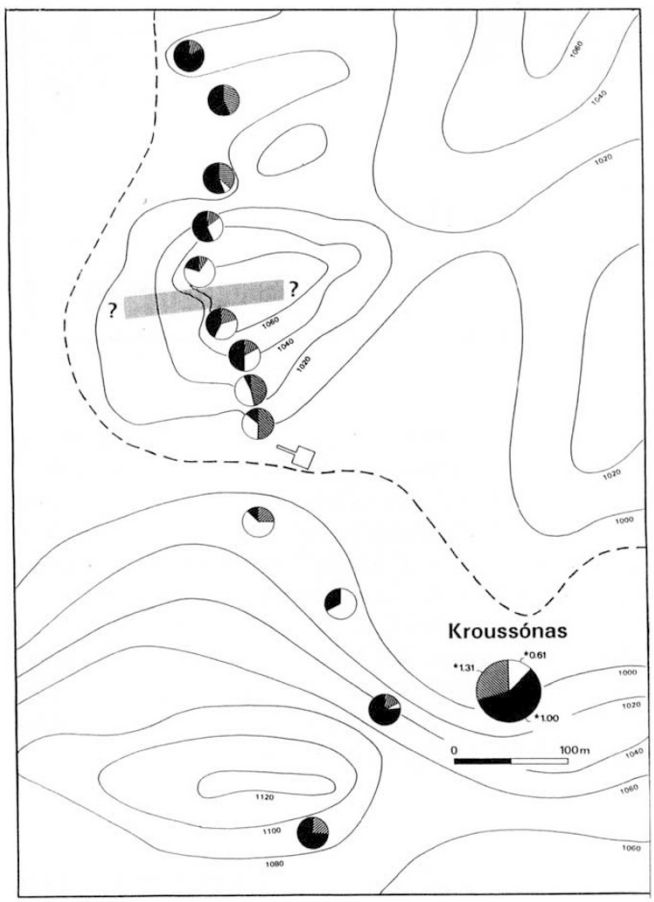

Fig. 3 Sketch of the Kroussónas site showing *sAat* allele frequencies. The stippled band indicates the approximate centre of the morphological hybrid zone. Altitudes are given in metres.

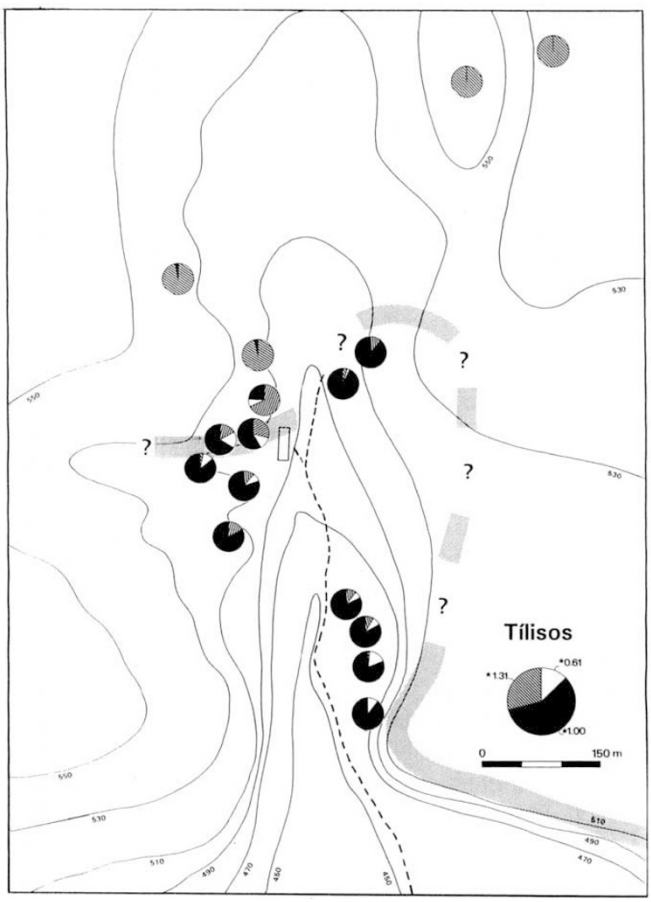

Fig. 4 Sketch of the Tílisos site showing *sAat* allele frequencies. The stippled band indicates the approximate centre of the morphological hybrid zone. Altitudes are given in metres.

studied electrophoretically (M. Schilthuizen, unpublished data). Given the fact that normally the chance of encountering this allele in an *A. hippolyti* sample is of the order of 5:70 = 7 per cent, the presence of the allele in 20 of 29 hybrid zone samples signifies the association with hybrid zones (χ^2-test, $P < 0.001$). The observation that both hybrid zones and various unconnected populations are characterized by the presence of the same electromorph, strongly suggests parallel evolution. Parallelism in hybrizymes has not been described before, although D. S. Woodruff (1989) hinted at the existence of a similar phenomenon in *Cerion* hybrid zones in the West Indies.

The origin of the hybrizyme allele is unknown but intracistronic recombination seems unlikely because the new allele is not found in all diallelic situations. At Kroussónas, it is only present in diallelic populations in the centre of the zone. Simple point mutation, as described by Bradley *et al.* (1993; see above) may well be the cause in this situation, too. Whatever its origin, the observed parallelism in this hybrizyme may possibly shed light on its maintenance in the hybrid zones.

The question of what maintains the hybrizyme alleles at such high frequencies in the (very narrow) hybrid zone is not easy to address. The parallelism may hint at a selective advantage of the hybrizyme. This advantage, however, would be strictly linked to the hybrid genetic background or to specific ecological circumstances present in the zone. The latter possibility is very unlikely because hybrid zones in *Albinaria* appear to be 'tension zones' (Key, 1968), the positions of which are not determined by exogenous selection (M. Schilthuizen, unpublished data). In fact, most authors (e.g. Barton *et al.*, 1983; Barton & Hewitt, 1985; D. S. Woodruff, 1989) have favoured a scenario in which hybrizymes are deleterious and are removed by selection against them. This selection pressure, however, is thought to be countered by the continuous generation of new, identical mutations, somehow triggered by an unbalanced genetic background (analogous to the release of mutator activity described by R. C. Woodruff *et al.* (1979) and R. C. Woodruff & Thomson (1980, 1982) in *Drosophila*). If this last hypothesis were to be correct, the question remains why the same hybrizyme would arise over and over again in the hybrid zone and why it would independently arise in two separate areas of hybridization? One possible solution to this problem would be that the number of metabolically active *sAat* allozymes is limited and that, with the *1.00 and *1.31 alleles as raw material for mutation to act on, the *0.61 allele is the only allozyme that can evolve.

On the basis of the mutation/selection hypothesis, as Barton *et al.* (1983) have pointed out, the strengths of the processes involved can be inferred from the spatial distribution of the hybrizymes. In the Kroussónas zone, the area in which hybrizymes are found is approximately 600 m wide. In the Tílisos zone, this area is narrower, approximately 200 m. These widths are similar to those of the clines in morphological and biochemical traits that characterize the hybrid zones. Therefore, the effective selection pressure s^* on the hybrizyme allele must be at least of the same order of magnitude as the s^* acting on the morphological and biochemical traits, i.e. $s^* = 8(\sigma/w)^2$ (Szymura & Barton, 1986), in which σ is the dispersal rate and w is the cline width. On the basis of data in Schilthuizen & Lombaerts (1994), the dispersal rate may be estimated at 3 m gen$^{-1/2}$. The resulting lower bounds for s^* are 2×10^{-4} at Kroussónas and 1.8×10^{-3} at Tílisos. With this lower limit for s^*, the respective maximum hybrizyme frequencies of 0.70 and 0.18 suggest mutation rates (μ) of at least 1.4×10^{-4} at Kroussónas and 3.2×10^{-4} at Tílisos. These figures correspond well with the mutation rate of $\geq 2.5 \times 10^{-4}$ estimated by Barton *et al.* (1983) for hybrizymes in *Podisma* grasshoppers. They are, however, at least an order of magnitude higher than those reported for enzyme loci in humans (Neel & Rothman, 1981). It should be stressed that the values presented here for s^* and μ are minimum values. The effective selection pressures are unlikely to be lower as the hybrizyme alleles would then be expected to have diffused over a wider area. Higher selection pressures (and accordingly higher mutation rates), on the other hand, are equally possible. Experimental hybridizations could provide direct measurement of the mutation rate.

The occurrence of hybrizymes well outside hybrid zones (see Fig. 1) requires further explanation. Similar observations have often been reported in hybrid zone studies. In fact, hybrizyme alleles are mostly referred to as 'alleles which are usually (i.e. outside hybrid zones) at frequencies of a few per cent or less' (Barton & Hewitt, 1985). If these allozymes are indeed identical to the hybrizymes, they might have arisen there by 'natural' mutation, i.e. not associated with hybridization. Their relatively high frequencies may have been brought about by genetic drift, given the very weak selection pressure on them.

The most important question concerning hybrizymes remains, of course, what is their evolutionary significance? If it were indeed true that mutation rates are increased in hybrids, then this may result in some hybrid disadvantage as mutations in general are deleterious (Alberts *et al.*, 1983) and the increased mutation rates will probably be felt across large parts of the

genome. Hybrizyme studies, therefore, may provide a window on the genetic processes underlying the evolution of reproductive isolation and, hence, speciation.

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