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RECENT APPLICATION OF AMINO-ACID RACEMIZATION-EPIMERIZATION TO THE DATING OF MIXED-AGE MARINE DEPOSITS OF CENTRAL AND SOUTHERN ITALY: AGE EVALUATION BASED ON THREE AMINO-ACID CONCENTRATIONS

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[1]C. S. per il Quaternario e l'Evoluzione Ambientale del CNR - Dip.to Scienze della Terra, Università "La Sapienza", Roma
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RIASSUNTO - *Recente applicazione della racemizzazione-epimerizzazione di amino acidi per la datazione di depositi marini di età mista in Italia Centrale e Meridionale: valutazione dell'età basata sulle concentrazioni di tre amino-acidi* - Il Quaternario *Italian Journal of Quaternary Sciences,* **9**(2), 1996, 661-666 - Vengono discussi i risultati in gran parte già pubblicati in precedenti lavori, ottenuti con il metodo basato sulla Epimerizzazione degli Aminoacidi (AAE). Sono stati identificati 4 depositi marini di età mista nelle aree di Montalto di Castro e di Tarquinia (Lazio): S. Agostino Nuovo (0.39±0.02; 0.48±0.02), Podere S. Pietro (0.38±0.02; 0.54±0.01), Lestra dell'Ospedale (0.48±0.02; 0.58±0.03) e Bandita S. Pantaleo (0.45±0.03; 0.59±0.04). Le analisi effettuate con l' ESR e con il $^{230}Th/^{234}U$ hanno evidenziato un singolo episodio marino. Gli altri depositi di età mista da noi identificati sono: S. Reparata in Sardegna (0.27±0.02; 0.38±0.02); Manca della Vozza, (0.50±0.02; 0.57±0.01) e Archi, (0.35±0.02; 0.49±0.01) in Calabria; Gallipoli in Puglia, (0.30±0.01; 0.41±0.04); Milazzo in Sicilia (0.29±0.02; 0.41±0.03) e Sapri in Campania (0.33±0.02; 0.09±0.02). A Manca della Vozza è stata effettuata una indagine $^{230}Th/^{234}U$ su due Cerastoderma campionati alla stessa quota (170 m s.l.m.) degli 8 *Glycymeris* esaminati con gli aminoacidi. I risultati (311+70,-46 ka e 191+24,-20 ka) sono in accordo con le nostre analisi e suggeriscono la presenza di un deposito di età mista. Nel deposito di Sapri sono state effettuate per la prima volta due datazioni radiocarbonio che segnalano anche in questo caso due episodi distinti (5510±100 BP e >40,000 BP), in accordo con le analisi AAE. Sono state misurate inoltre le concentrazioni dei tre aminoacidi leucina, D-alloisoleucina, acido α-amino-N-butirrico. I rispettivi valori, inseriti in un diagramma ternario, individuano "campi" cronologici in accordo con le età stimate in base al rapporto aile/ile o misurate con altri metodi di datazione. Questo approccio metodologico fornisce importanti informazioni su parametri cinetici e si mostra molto promettente per stimare età di campioni del Quaternario.

ABSTRACT - *Recent application of amino-acid racemization-epimerization to the dating of mixed-age marine deposits of Central and Southern Italy: Age evaluation based on three amino-acid concentrations* - Il Quaternario *Italian Journal of Quaternary Sciences,* **9**(2), 1996, 661-666 - In this study, we rely on the ratio of D-alloisoleucine to L-isoleucine (aile/ile) in the genera *Glycymeris* collected from Pleistocene and Holocene mixed-age marine units. Amino Acid Epimerization (AAE) results are in good agreement with those obtained for the same sediments by $^{230}Th/^{234}U$, and ^{14}C. In the Montalto di Castro and Tarquinia areas, Latium (Central Italy), four marine mixed-age deposits were identified. Sampled sites are: S. Agostino Nuovo, Podere S. Pietro, Lestra dell'Ospedale and Bandita S. Pantaleo. The values obtained in these four deposits by ESR and $^{230}Th/^{234}U$ methods indicate a marine episode. The other mixed-age deposits identified are: S. Reparata in Sardinia; Manca della Vozza and Archi in Calabria (Southern Italy); Gallipoli in Apulia (Southern Italy; Milazzo in Sicily and Sapri in Campania (Southern Italy). From Manca della Vozza, a $^{230}Th/^{234}U$ dating was also performed. The analysis, which was carried out on *Cerastoderma lamarcki cotronensis* samples found at the same elevation (170 m a.s.l.) as the 8 *Glycymeris insubricus* samples, are in good agreement with those obtained by AAE, and put to evidence the mixed-age deposit. Most of AAE results have been published in previous works. From the Sapri deposit, which is examined for the first time in this paper with the Radiocarbon method, two analyses were made on the same *Glycymeris* spp. samples analyzed with AAE. In this deposit the radiocarbon dates also put to evidence two marine episodes, which are in good agreement with those obtained with AAE. Finally, the three component analysis technique provides important information on kinetic parameters and can be used to estimate the ages of Pleistocene to Holocene mollusks.

Key-words: Racemization-epimerization dating, mixed-age marine deposits, Central and Southern Italy
Parole chiave: Datazione, racemizzazione-epimerizzazione degli aminoacidi, depositi marini di età mista, Italia centrale e meridionale

1. INTRODUCTION

During the last 25 years, a method for dating fossil samples using amino acid racemization-epimerization reaction has been developed. The method has an effective dating range beginning at a few thousand years BP and extending to several hundred thousand years (the Pleistocene), the actual range being dependent upon the general temperature of the region where the sample was found. Only few grams (1-2) of fossil material are required for the analysis. Racemization-epimerization dating is based on the fact that the molecules of amino acids in active living tissues are of the L-isomer variety, that is the only form used by animal enzyme systems. When an animal dies or the tissue ceases to be metabolically active (*e.g.* tooth enamel), molecules begin to turn into the D-isomer variety. The process continues until the ratio of the two isomers is 1:1. The rate of racemization-epimerization is uniform and specific for each amino acid. Since racemization-epimerization is a chemical reaction, it is dependent on temperature. Thus, in order to date a fossil sample using this method, it is necessary to evaluate the average temperature to which the fossil has been exposed.

However, this temperature evaluation can be eliminated using a procedure in which the *in situ* rate of amino acid racemization for a particular area is calculated by measuring the extent of racemization-epimerization in a sample dated with another method (^{14}C, $^{230}Th/^{234}U$, ESR etc.). After this calibration has been carried out, other samples from the general area can be dated, based on their extent of amino acid racemization. The

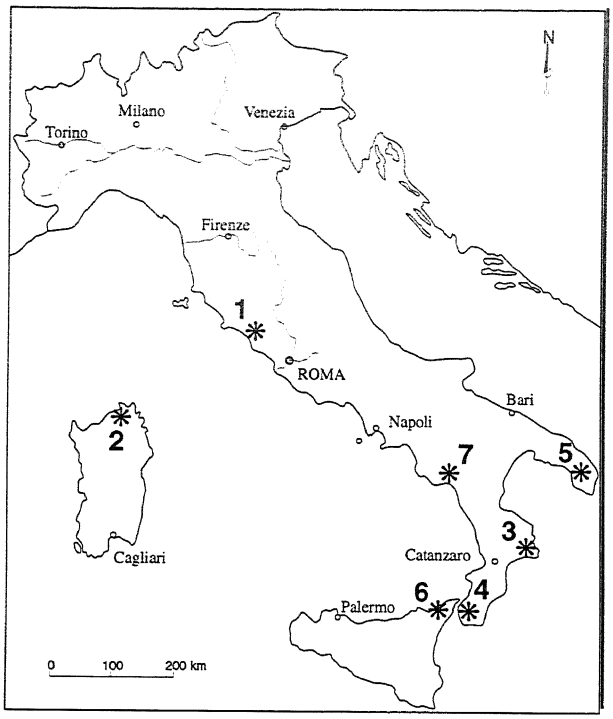

Fig. 1 - Locations of mixed-age marine deposits (see Table 1).
Ubicazione dei depositi marini di età mista i cui nomi sono indicati nella Tabella 1.

amino acid racemization-epimerization reaction is an important chronological method with applications in geology, anthropology and palaeontology.

Amino acid racemization-epimerization analyses have traditionally been used primarily for correlation and dating of Pleistocene marine units. In this study, two other general types of applications are discussed: 1) characterization of mixed-age marine deposits, and 2) age evaluation based on three amino acid concentrations: leucine, D-alloisoleucine, α-amino-N-butyric acid.

As for the geological outline of the areas here studied, see: Ambrosetti *et al.* (1981), Bartolini *et al.* (1984), Bosi *et al.* (1990), Conato & Dai Prà (1980), Hearty & Dai Prà (1986) for Tarquinia and Montalto di Castro area; Belluomini *et al.* (1986) for Sardinia; Belluomini *et al.* (1988) for the Manca della Vozza area; Brancaccio *et al.* (1990) for the Sapri area.

2. RESULTS AND DISCUSSION

The reaction used in this study, concerning *Glycymeris* fossils collected from Pleistocene and Holocene mixed-age marine units, is the isoleucine epimerization reaction, for which the equilibrium ratio of D-alloisoleucine to L-isoleucine (aile/ile) is determined to be 1.3 in mollusks (Schröeder & Bada, 1976). The results are in good agreement with those obtained for the same sediments by $^{230}Th/^{234}U$, ESR and ^{14}C. In the Montalto di Castro and Tarquinia areas (Belluomini *et al.*, 1993) in Latium (Central Italy) (Fig. 1, Table 1), 4 mixed-age marine deposits were identified. Sampled sites are: S. Agostino Nuovo (0.39±0.02; 0.48±0.02), Podere S. Pietro (0.38±0.02; 0.54±0.01), Lestra dell'Ospedale (0.48±0.02; 0.58±0.03) and Bandita S. Pantaleo (0.45±0.04; 0.59±0.04). As shown in Table 1, the values obtained in these 4 deposits with $^{230}Th/^{234}U$ and ESR methods recognize only one of two marine episodes.

The other mixed-age deposits identified are (Fig. 1, Table 1): S. Reparata, Sardinia, (0.27±0.02; 0.38±0.02) (Belluomini *et al.*, 1986); Manca della Vozza, (0.50±0.02; 0.57±0.01) (Belluomini *et al.*, 1988) and Archi, (0.35±0.02; 0.49±0.01) (Hearty *et al.*, 1986), Calabria, Southern Italy; Gallipoli, Apulia, Southern Italy, (0.30±0.01; 0.41±0.04); Milazzo, Sicily, (0.29±0.02; 0.41±0.03) (Hearty *et al.*, 1986; Belluomini, 1985) and Sapri, Campania, Southern Italy (0.33±0.02; 0.09±0.02).

From Manca della Vozza, a $^{230}Th/^{234}U$ investigation was also performed. The analysis, which was made on two samples of *Cerastoderma lamarcki cotronensis* found at the same elevation (170 m a.s.l.) as the 8 *Glycymeris* samples, gave the following ages: 311^{+70}_{-46} ka and 191^{+24}_{-20} ka. The two latter results are in agreement with those obtained by amino acid racemization-epimerization and evidence the mixed-age of the marine deposit. From the Sapri deposit, studied for the first time

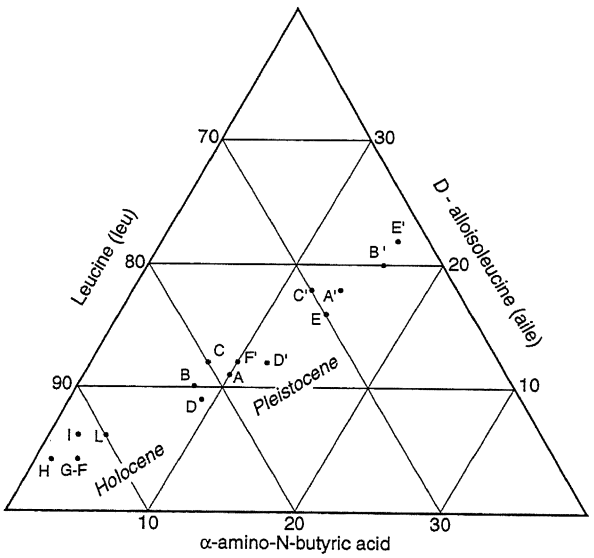

Fig. 2 - Leucine – D-alloisoleucine – α-amino-N-butyric acid diagram of fossil shell samples from raised marine deposits. Coupled points (*e.g.* A-A') belong to the same mixed-age deposit.For the 3 component values, see Table 2.
Diagramma ternario (Leucina, D-alloisoleucina, acido α-amino-N-butirrico) relativo a molluschi fossili campionati in depositi marini emersi. La distribuzione dei punti nel diagramma individua aree corrispondenti a distinte fasce cronologiche che vanno dal Pleistocene all'Olocene. Le coppie di lettere uguali (ad es. A-A') indicano i depositi misti costituiti da fossili di età diversa. Le concentrazioni dei tre aminoacidi scelti sono riportate in Tabella 2.

with the radiocarbon method, two analyses were made on the same *Glycymeris* samples analyzed using the epimerization method. Radiocarbon dates: 5510±100 BP and >40,000 BP indicate two marine episodes in agreement with the ages obtained with the epimerization method.

Table 1 / *Tabella 1*

Site and reference in Figure 1	Elev. (m a.s.l.)	*Glycymeris* spp. $\bar{x} \pm \sigma$ (n)[a]	AAE Age (ka)[b]	ESR Age (ka)	$^{230}Th/^{234}U$ Age (ka)	^{14}C Age (years BP)
Sant'Agostino Nuovo (1)	14	0.39±0.02 (2)	~120	163 ÷ 178(d)	156±12(d)	
		0.48±0.02 (12)	~200			
Podere San Pietro (1)	25	0.38±0.02 (6)	~120	102 ÷ 118(e)	99+18,-6; 115+15,-13(e)	
		0.54±0.01 (2)	~200			
Lestra dell' Ospedale (1)	45	0.48±0.02 (13)	~200	211±50(e)	200+56,-20(e)	
		0.58±0.03 (20)	~300			
Bandita San Pantaleo (1)	55	0.45±0.03 (4)	~200	202 ÷ 259(c)	212 ± 38(c)	
		0.59±0.04 (13)	~300			
Santa Reparata (2)	1	0.27±0.02 (3)	~70			
		0.38±0.02 (6)	~120			
Manca della Vozza (3)	170	0.50±0.02 (4)	~200		191+24,-20(f)	
		0.57±0.01 (4)	~300		311+70,-46(f)	
Archi (4)	110	0.35±0.02 (7)	~120			
		0.49±0.01 (4)	~200			
Gallipoli (5)	4	0.30±0.01 (7)	~100			
		0.41±0.04 (12)	~120			
Capo Milazzo (6)	55	0.29±0.02 (9)	~100			
		0.41±0.03 (2)	~120			
Sapri (Limito) (7)	15	0.33±0.02 (14)	~100			>40,000
		0.09±0.02 (3)	<10,000			5510±100

(a) aile/ile (D-alloisoleucine/L-isoleucine) ratios are given as mean ($\bar{x}$), standard deviation (σ) and number of shells analyzed (n).

(b) The calculated epimerization ages have an uncertainly of ± 10-15%.

(c) Radtke *et al.* (1982); (d) Radtke *et al.* (1983); (e) Radtke (1983); (f) Belluomini *et al.* (1988).

For age evaluation based on leucine, D-alloisoleucine and α-amino-N-butyric acid concentrations involving the utilisation of three components (Fig. 2, Table 2), we used the stable amino acid leucine and the products of two amino acids diagenetic reactions, D-alloisoleucine from L-isoleucine epimerization and α-amino-N-butyric acid, produced from the dehydration of L-threonine.

For bivalve mollusk shells from raised marine deposits from temperate environments of the Mediterranean basin, the 3-component analysis yields a linear relationship (Belluomini *et al.*, 1993). Although the shells came from widely dispersed sites, and thus their average exposure temperatures are different as are other environmental parameters (*e.g.* soil pH, humidity), these factors apparently do not greatly affect the position of similar age samples on the plot.

This implies that the activation energies for isoleucine epimerization and threonine dehydration must be similar; otherwise, with increasing temperature, we would expect to see a significant displacement from the linear relationship (Bada & Belluomini, 1987). Based on the estimated and determined ages of the samples, we have made a preliminary age subdivision of the zones on the diagram (Belluomini *et al.*, 1993); thus, in this paper we have plotted the three amino acid concentrations of Podere S. Pietro (A, A'), Lestra dell'Ospedale (B, B'), Bandita S. Pantaleo (C, C'), Santa Reparata (D, D'), Manca della Vozza (E, E'), Sapri (Limito) (F, F') and

Table 2 / *Tabella 2.*

Site	Ref. in Fig. 2	α-ABA %	aile %	leu %	aile/ile	AAE Age (ka)	Radiocarbon Age (yrs BP)	
Podere San Pietro[a]	A	10	11	79	0.38	0.1		
" " "	A'	14	18	68	0.54	0.2		
Lestra dell' Ospedale[a]	B	8	10	82	0.48	0.2		
" " "	B'	16	20	64	0.58	0.3		
Bandita San Pantaleo[a]	C	8	12	80	0.45	0.2		
" " "	C'	12	18	70	0.59	0.3		
Santa Reparata[b]	D	9	9	82	0.27	0.07		
" "	D'	12	12	76	0.38	0.1		
Manca della Vozza[c]	E	14	16	70	0.50	0.2		
" " "	E'	16	22	62	0.57	0.3		
Sapri (Limito)	F	3	4	93	0.09	Holocene	5510±100	(R-2256)*
" "	F'	10	12	78	0.33	0.1	>40,000	(R-2257)
Pego[a]	G	3	4	93	0.12		5710±100[b]	(R-2013)
Doñana - D 18[a]	H	1	4	95	0.05		10,130±65[b]	(R-2183)
Cadiz - C 5[a]	I	2	6	92	0,10		2270±50[b]	(R-2181)
Doñana 9[a]	L	4	6	90	0.08		1860±50[b]	(R-2185)

[a] Belluomini *et al.*, 1993; [b] Belluomini *et al.*, 1986; [c] Belluomini *et al.*, 1988.
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some Holocene sites of Spain (*e.g.* Pego, Doñana-D 18, Doñana-9 and Cadiz-C5) on the diagram. As shown in Figure 2, the age zones indicate that the three component technique can be used to directly estimate the ages of shells ranging from Pleistocene to Holocene in good agreement with epimerization age values.

3. CONCLUSIONS

Amino acid epimerization method showed to be very useful to characterize the mixed-age marine deposits because the analyses can usually be carried out on individual shells (analyses require only 1-2 gr of shell) and, therefore, a large number of samples can be dated from a particular deposit in order to reconstruct their age distribution. The analyses have shown that in several deposits of Central and Southern Italy redeposited shells are quite common. Finally, the three component analysis technique also provides important kinetic parameters informations and it can be used to estimate the ages of Pleistocene to Holocene mollusks.

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