再 刊 編 號：3
Reprint No. 3

抽印自　中華民國貝類學報
第十三卷
Reprinted from
Bulletin of Malacology, ROC
Vol. 13, pp. 1-7, 1987

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文蛤資源研究
I．同功異構　的應用初報

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Malacological Research on *Meretrix* Resources in Taiwan
I. Preliminary Report on Isozyme Study

Wen-Lung Wu and Hsiu-Ping Liu

農委會補助計畫編號：
76農建-8.1-漁-37AB

Bull. Malacol. R.O.C. 貝類學報 13 : 1 – 7 (1987)

文蛤資源研究

I. 同功異構酶的應用初報

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關鍵詞：雙殼綱—簾蛤科，同功異構酶，文蛤，電泳

Key words: Bivalvia-Veneridae, Isozyme, *Meretrix*, Electrophoresis.

前言

從事資源研究管理時，鑑定捕獲對象的分類地位，是首要的條件。例如捕獲對象若是由"單位資源"(由幾個stock所組成)而來，對其中一個小集團施予漁獲壓力並不會影響其他小集團；但是漁獲對象只是由"單一資源"(由一個stock所組成)而來，則對其施予漁獲壓力時則會對資源有所影響(Kubo and Yoshihara 1969)。因此唯有正確判定漁獲對象的分類地位，才能訂出正確的漁業資源管理政策，也才能使資源獲得最佳的合理利用。

一般常用的族群判別的方法有形態學、生態學、寄生蟲學、血清學以及漁況學等方法。這些方法用以判定族群各有困難存在。本文利用生化學中的電泳法，希望藉此結果來判定種別，定出族群，並進而探討其與環境因子間的關係。

由生物學上對"種"的定義可知，種與種間具有生殖隔離的現象，具有固定的對偶基因差異(Fixed allelic difference)，而生物學上對"族群"的定義可知族群爲同一種內非逢機的生殖群體，具有明顯的對偶基因頻度差異(Allelic frequency difference)，因此藉着生化電泳的分析即可判定種別及定出族群。

材料與方法

(一)材料—由鹿港與淡水兩地採得的鮮活文蛤帶回實驗室立即採下所需的組織，計有消化腺(digestive gland)，足部肌肉(Foot)，鰓(gill)，前後閉殼肌(anterior / posterior adductor muscle)以及外套膜(mantle)。

(二)電泳有關材料的製備—新鮮組織取下後，加入雙倍量的萃取液(Extraction buffer-Tris, EDTA, NADP)，再以超音波研磨器(Sonicator)將組織研磨，細胞打破，促使同功異構酶(Isozyme)釋放於萃取液中，再放入冷凍離心機中以15,000 rpm離心30分鐘，取其上層清液再分裝於－70℃超低溫冰箱中儲存備用。12% starch gel(Divall 1984)放於三角錐瓶內，一邊加熱一邊搖動，直到starch完

全溶解呈透明粘稠狀，把多餘氣泡趕走，倒入模型中，置於冰箱中冷却備用。用Whatman No.1 filter paper 0.7×0.9 mm吸取離心後之上層清液，插於gel上。放入電泳槽中，利用Net charge之不同，使同功異構酶分離。電泳跑完後，取出gel，以水平方向切成3～4片，置於染色盒中，加以染色。

(三)緩衝液系統—本次實驗採9種 buffer system,係修改Shaw and Prasad (1970)的方法而來：

a. Electrode buffer: 135 mM tris, 43 mM Citric acid, pH 7.0.
   Gel buffer: 9 mM tris, 2.9 mM Citric acid, pH 7.0.
b. Electrode buffer: 250 mM tris, 50 mM Citric acid, pH 8.0.
   Gel buffer: 9.6 mM tris, 1.9 mM Citric acid, pH 8.0.
c. Electrode buffer: 100 mM tris, 100 mM maleic acid, 10 mM EDTA, 20 mM $MgCl_2 \cdot 6H_2O$, pH 7.4.
   Gel buffer: 10 mM tris, 10 mM maleic acid, lmM EDTA, 2mM $MgCl_2 \cdot 6H_2O$, pH 7.4.
d. Electrode buffer: 40 mM Citric acid (adjust pH with N–(3–aminopropyl)–morpholine), pH 6.1.
   Gel buffer: 2 mM Citric acid (adjust pH with N–(3–aminopropyl)–morpholine), pH 6.1.
e. Electrode buffer: 129 mM tris, 3 mM EDTA, 70 mM boric acid, pH 8.6.
   Gel buffer: 45 mM tris, 1mM EDTA, 25 mM boric acid, pH 8.6.
f. Electrode buffer: 200 mM Sodium acetate (adjust pH with acetic acid), pH 5.6.
   Gel buffer: 1:22 dilution of electrode buffer.
g. Electrode buffer: 30 mM Lithium hydroxide, 190 mM boric acid, pH 8.1.
   Gel buffer: 1:9 mixture of stock A and B.
   stock A – 30 mM Lithium hytroxide, 190 mM boric acid, pH 8.1.
   stock B – 50 mM tris, 8 mM Citric acid, pH 8.4.
h. Electrode buffer: 135 mM tris, 43 mM Citric acid, pH 8.0.
   Gel buffer: 5 mM Histidine, pH 8.0.
i. Electrode buffer: 300 mM boric acid, 60 mM NaOH, pH 8.2.
   Gel buffer: 76 mM tris, 5 mM Citric acid, pH 8.7.

(四)電泳的Voltage, time及stain substrate:

a. Phosphohexose isomerase (Pgi) – buffer system c, 100 v, 16 hr., fructose-6-phosphate.
b. Phosphoglucomutase (Pgm) – (a) buffer system c, 100 v, 16 hr., glucose-1-phosphate, (b) buffer system e, 300 v, 8 hr., glucose-1-phosphate.
c. Isocitrate dehydrogenase (Idh) – buffer system a, b, e, f, g, i, 20 v/cm, 5hr., Sodium-isocitrate.
d. Malate dehydrogenase (Mdh) – buffer system a, b, c, d, e, f, g, h, 12.5 v/cm, 4 hr., Sodium-malate.
e. Aminoaspartate transferase (Aat) – buffer system a, b, c, d, e, f, g, h, i, 25 v/cm, 18 hr., Aspartic acid, α-ketoglutarate, pyridoxial-5-phosphate, fastblue-BB-salt.
f. 6-phosphagluconate dehydrogenase (6-Pgdh) – buffer system a, b, c, d, e, f, g, h, i, 25 v/cm, 5 hr., 6-phosphogluconia acid.
g. Leucine aminopeptidase (Lap) – buffer system c, d, e, f, g, h, i, 12.5 v/cm, 6 hr., L-leucyl-β-naphthylamide.
h. Adenylate kinase (AK) – buffer system e, 300 v, 8 hr., D-glucose, Hexokinase, ADP.
i. Acid phosphatase (Acp) – buffer system i, 220 v, 7 hr., α-naphthyl acid phosphate.

j. Creatine kinase (CK) – buffer system e, 300 v, 8 hr., D-glucose, hexokinase, ADP, phosphocreatine.
k. Malic enzyme (ME) – buffer system e, 300 v, 8 hr., Sodium-L-malate, pH 7.0.
l. Glucosephosphate isomerase (Gpi) – buffer system e, g, 300 v, 8 hr., Fructose-6-phosphate.

結果

在九種緩衝液系統及十二種同功異構酶研究中，發現有些同功異構酶的分離及染色效果不理想，而有些則没有多型的現象，只有Pgi(Fig.1)，Pgm(Fig.2)，Aat (Fig.3)，Mdh(Fig.4)及 Idh(Fig.5)，它們不但分離染色效果好，而且具有 Polymorphism的現象，因此爾後即可利用這五種同功異構酶的對偶基因頻度，用以判定種別，定出族群，並進而可以討論其與環境因子間的關係。

在對偶基因命名時，我們通常以最常見的對偶基因訂爲100，其餘的對偶基因再依其相對的移動率而訂出。本文以 Pgi 的結果爲例，訂出有150，100以及60三種 alleles (表一)。鹿港區的Allele frequency 在100時爲0.7805；而淡水區的Allele frequency在100時則爲0.5292。兩地的Alleles frequency 再經χ^2檢定其是否符合 Hardy – Weinberg equilibrium hypothesis，結果如表二，表三所示。在鹿港區，Heterozygosity 的期望值$H_L(exp.)=1-\Sigma Xi^2=0.378$(表一)，而由實際所得的觀察值(obs.)爲0.395，兩者甚爲相近。經由表二所得$\chi^2=7.02^{NS}$ < P (0.01,df=3)=7.81，因此鹿港標本的 Pgi的Allele frequency處於 Hardy–Weinberg equilibrium state。然而在淡水區，

Table 1. Alleles frequency from Dec. 1986 to July 1987 in Lukang and Tanshui area

Alleles Freq.	Lukang	Tanshui
150	0.0955	0.3149
100	0.7805	0.5292
60	0.0573	0.0877
	$H_L(exp.)=0.378$	$H_T(exp.)=0.613$

Table 2. Chi-square test of alleles frequency from Dec. 1986 to July 1987 in Lukang area

Alleles Freq.	Obs.	Exp.	(O–E)	$(O-E)^2$	$(O-E)^2/E$
(150, 150)	1	1.43	–0.43	0.18	0.13
(150, 100)	23	23.40	–0.40	0.16	0.01
(150, 60)	5	1.72	3.28	10.76	6.25
(100, 100)	94	95.59	–1.59	2.53	0.03
(100, 60)	13	14.04	–1.04	1.08	0.08
(60, 60)	0	0.52	–0.52	0.27	0.52
				Sum	7.02

$\chi^2=7.02^{NS}$ < P(0.05, df=3) = 7.81

Table 3. Chi-square test of alleles frequency from Dec. 1986 to July 1987 in Tanshui area.

Alleles Freq.	Obs.	Exp.	(O−E)	$(O-E)^2$	$(O-E)^2/E$
(150, 150)	24	15.27	8.73	76.21	4.99
(150, 100)	33	51.33	−18.33	335.99	6.55
(150, 60)	5	8.51	−3.51	12.32	1.45
(100, 100)	54	43.13	10.81	118.16	2.74
(100, 60)	12	14.29	−2.29	5.24	0.37
(60, 60)	5	1.18	3.82	14.59	12.37
				Sum	28.47

$x^2 = 28.47\ ^{**} > P(0.01, df=3) = 11.34$

Heterozygosity 的期望值H_T(exp.)$=1-\Sigma Xi^2=0.613$(表一)，而由實際所得的觀察值(obs.)爲 0.4610，兩者差異甚大。再由表三得到 $x^2=28.47^{**} > P(0.01, df=3) = 11.34$，所以淡水區標本的Pgi allele frequency有偏離Hardy-Weinberg equilibrium state而且具有 Heterozygosity deficient 現象。

討論

Skibinski *et al.*, 1977 研究淡菜發現應用39個loci，結果只有13個loci有多型的現象。Morton and Britton (1986) 也對*Corbicula*的多型現象作了詳細描述。我們從事文蛤電泳研究時，發現以TC, pH7.0來分離 Mdh 及 Idh 時均沒有多型現象；但是以Citric acid，pH 6.1來分離 Mdh 時則可將 Cytoplasmic 以及 Mitochondria 的loci分離得很清楚。同樣地分離 Idh 時亦可將其Cytoplasmic以及mitochondria的loci分離開來。因此有些研究結果顯示出多型性很低甚至沒有，是值得進一步檢討與考慮的。

在許多族群遺傳研究中，有關自然族群遺傳多型性受到Selection的情形，以*Mytilus edulis*的 Lap polymorphism 以及其與環境塩度關係的研究最爲詳盡(Koehn *et al.*, 1976; Koehn 1977, 1978; Koehn *et al.*, 1980)。Lap已被證實在*Mytilus*中與塩度蓄積有關(Koehn 1978; Young *et al.*, 1979; Moore *et al.*, 1980)，其他軟體動物也有類似的結果(Garthwaite 1986)。不同的 Lap genotype的*Mytilus*也已被證實能影響其相對適應能力(Koehn *et al.*, 1980; Koehn and Gaffney 1984)。我們在淡水地區所做 Pgi 的 x^2-Test發現有偏離Hardy-Weinberg Law現象，而且具有 Heterozygosity deficient 現象，然而在鹿港地區則無此現象，是否意味着淡水地區處於河口海域，而鹿港地區則位於沿海，兩者的塩度環境不同而造成有如此的差異，值得進一步探討其與環境因子相關的比較，希望將來能够找出更適當的基因型，以便應用於將來有關文蛤

放流計劃中，以提高幼苗的活存率，期望資源量增加。

謝辭

作者感謝中央研究院動物研究所李信徹博士提供有關電泳實驗的設備與方便，同時也感謝研究助理蔡昌明，趙維誠以及台大漁科所研究生戴仁祥的協助，一併感謝。部份經費獲得行政院農委會專案（76農建—8.1漁—37AB）補助，謹此致謝。

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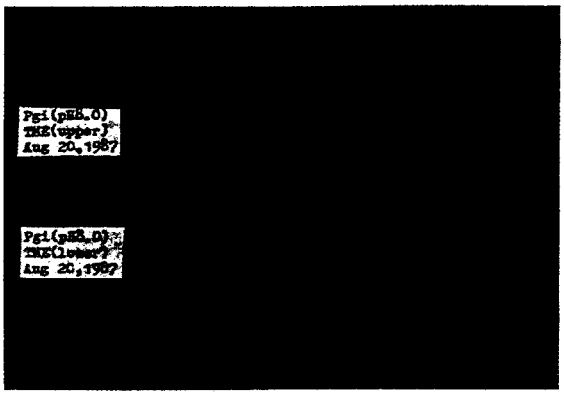

Fig. 1. Pgi alleles in Tris-Maleic-EDTA buffer system.

Fig. 2. Pgm alleles in Tris-Maleic-EDTA buffer system.

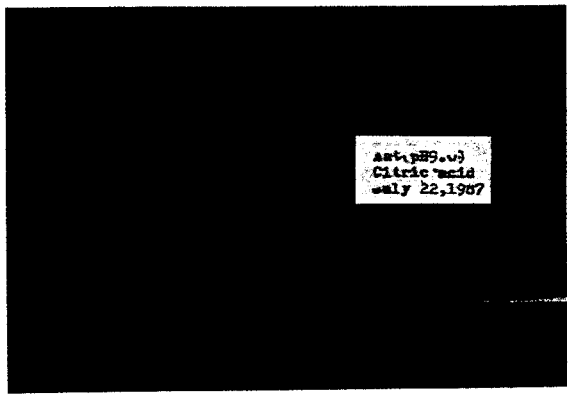

Fig. 3. Aat alleles in Citric acid buffer system.

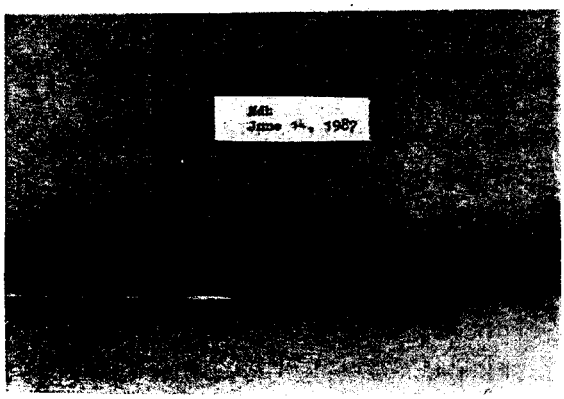

Fig. 4. Mdh alleles in Citric acid buffer system.

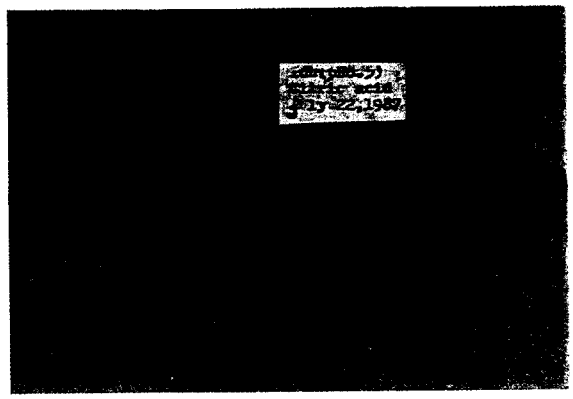

Fig. 5. Idh alleles in Citric acid buffer system.

Malacological Research on *Meretrix* Resources in Taiwan I. Preliminary Report on Isozyme Study

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The authors use allozyme variation to distinguish species and populations of *Meretrix* in Taiwan area, and to discuss the relationship between heterozygosity deficient and environmental factors.

Allozyme variation of *Meretrix* in Lukang and Tanshui has been compared by starch gel electrophoresis. On the basis of using nine different buffer systems, 12 isozymes were used to investigate allozyme variation. When Pgi, Pgm in citric acid buffer system and Idh, Aat, and Mdh in Tris-Maleic-EDTA buffer system were employed, we found that the separation and stain results are effective. Therefore, these five isozymes are used to do further studies.

Pgi allele frequency in Lukang and Tanshui by using Chi-square test, and the calculated value based on Hardy-Weinberg expectations is identical with the observed value in Lukang, but is inconsistent in Tanshui.