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SHORT COMMUNICATION

Genetic subdivision of the upwelling copepod *Calanoides carinatus* (Krøyer, 1849) off the continental shelf of Ghana

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This study has shown that the two stocks of Calanoides carinatus appearing off the shelf of Ghana during the upwelling season are genetically different. Also, the gene Gpi[a] could probably be a marker for their subdivision.

Calanoides carinatus occurs in the South Atlantic Central Waters (SACW), as diapausing stage V copepodites, below 200 m depth. From there, they are transported northwards by oceanic subsurface currents to the continental slope southwest of the British Isles, and even as far as 60°N. However, they are not known to reproduce in northern latitudes (Williams and Conway, 1988; John *et al.*, 1998). When the SACW is upwelled off the coast of West Africa during the upwelling season from July to September, a phenomenon that is not well understood (Bakun, 1978), they moult into adults and subsequently reproduce several generations, which develop very rapidly (Valentin *et al.*, 1987; Kuipers *et al.*, 1993; Postel *et al.*, 1995). It takes 7 days for the eggs to develop to stage IV copepodites, which then reach sexual maturity within 7–12 days. It takes a further ~14 days for the mature females to spawn and then perish soon afterwards (Mensah, 1974). Peterson and Painting similarly observed that, in the laboratory, development from egg to maturity takes 18.3 days at a temperature of 15.5°C and 12 days at 19.5°C (Peterson and Painting, 1990).

Mensah observed that the stage V copepodites of *C. carinatus* arrive on the Ghanaian shelf as two different stocks (Mensah, 1974). He found that the interval between the end of spawning of the first-generation females from the first stock and the commencement of spawning of the first-generation females from the second stock was 7 days in 1970 and 6 days in 1971. In 1970, the first stock produced two complete generations, while the second stock produced only one. In the following year, the upwelling period was prolonged and both stocks reproduced two complete generations.

The end of the upwelling season is marked by an increase in sea surface temperature and migration of *C. carinatus* into deeper waters offshore, where they enter the diapause stage until the next season. The slow ripening of the gonads is the only sign of development during the diapause (Kosobokova *et al.*, 1988; Timonin *et al.*, 1992; Arashkevich *et al.*, 1996; Arashkevich and Drits, 1997).

It is not known whether there is any genetic differentiation between the two stocks of *C. carinatus* that arrive at the Ghanaian shelf during the major upwelling (Mensah, 1974). The objective of this study was to examine specific enzyme systems that could provide the basis for differentiating the two stocks.

Three sets of zooplankton samples were collected during the upwelling season (31 July, 6 August and 12 August 1999) from a station (05°32′11″; 0°06′38″) ~14 miles off the coast of Tema, Ghana. A standard WP-2 net of 200 μm mesh size was hauled from a depth of 50 m to the surface. About a quarter of each haul was preserved in 70% ethyl alcohol for identification of developmental stage of *C. carinatus*. The remainder was immediately frozen in liquid nitrogen and later stored at –80°C in the laboratory.

All the samples comprised adult stages, but the second

sample included stage I–III copepodites as well. Hence, it was assumed that adults from the first two samples belonged to two different stocks, and mature females from each sample (i.e. female subsamples) were, therefore, selected for comparative study. Enzyme–genetic techniques were applied, since they afford optimum results in this kind of investigation (Parker *et al.*, 1998; Sunnucks, 2000). Three enzymatic systems, known for their high degree of polymorphism in *Calanus* spp., were examined (Sywula *et al.*, 1993): esterase (EST; EC 3.1.1), glucose-phosphate isomerase (GPI; EC 5.3.1.19) and leucine aminopeptidase (LAP; EC 3.4.11.1). Homogenates of randomly selected mature females were subjected to electrophoresis on cellulose acetate, using recommended staining techniques (Hebert and Beaton, 1989). Each specimen from the 120 specimens of each subsample was examined with respect to all three enzymatic systems. Alleles were designated according to the diminishing electrophoretic mobility of the relevant proteins. As expected, the esterases were coded by a series of loci, but only one of them, the locus whose expression products were characterized by the highest electrophoretic mobility, could be interpreted genetically without recourse to special breeding experiments. However, in some specimens it was not possible to interpret genetically all three electrophoretic phenotypes.

Each subsample was tested for agreement with Hardy–Weinberg proportions using the χ^2 test. The statistical significance of the differences in allele frequencies between the two subsamples was estimated using the G^2 test (Adam, 1987). The electrophoretograms obtained were in agreement with the model predicted for a dimeric protein coded by a single locus with six alleles in the case of *Gpi*, and a monomeric protein coded by a single locus with four alleles in the case of *Est-1* and with three alleles in the case of *Lap*.

The results indicate that all the loci examined were polymorphic, with a considerable agreement between the observed and expected frequencies of genotypes in the subsamples (Table I). As regards quantitative differences, in the case of the *Gpi* locus, the frequencies of alleles *c* and *e* did not differ significantly. However, the pooled frequencies of alleles for which the value of G^2 could be calculated were statistically significant ($G^2 = 3.8746$; $P < 0.05$). With respect to the *Est-1* locus, the allele frequencies differ significantly, and with regard to the *Lap* locus, the difference was highly significant ($G^2 = 23.1132$; $P < 0.001$).

The alleles Gpi^a, $Est\text{-}1^d$ and Lap^c were not found in subsample I, and with respect to the qualitative difference between the subsamples, that for Gpi^a was highly significant (Table II). The electrophoretic phenotypes containing the

Table I: Genetic comparison of two subsamples assumed to belong to different populations

Genotype	Subsample I		Subsample II		Genotype	Subsample I		Subsample II	
	O	E	O	E		O	E	O	E
Gpi^{aa}	–	–	–	0.28	Gpi^{ef}	1	0.36	–	0.07
Gpi^{ab}	–	–	–	1.06	Gpi^{ff}	–	0.04	–	0.01
Gpi^{ac}	–	–	–	0.35	$Est\text{-}1^{aa}$	–	0.02	–	0.00
Gpi^{ad}	–	–	10	8.53	$Est\text{-}1^{ab}$	–	0.02	–	0.10
Gpi^{ae}	–	–	1	0.40	$Est\text{-}1^{ac}$	1	0.97	1	0.88
Gpi^{af}	–	–	–	0.10	$Est\text{-}1^{ad}$	–	–	–	0.01
Gpi^{bb}	1	0.60	3	1.01	$Est\text{-}1^{bb}$	1	0.06	1	1.06
Gpi^{bc}	2	1.28	–	0.67	$Est\text{-}1^{bc}$	3	4.86	17	18.58
Gpi^{bd}	13	11.78	15	16.27	$Est\text{-}1^{bd}$	–	–	–	0.20
Gpi^{be}	–	1.43	–	0.77	$Est\text{-}1^{cc}$	108	107.09	82	81.38
Gpi^{bf}	–	0.30	–	0.19	$Est\text{-}1^{cd}$	–	–	2	1.77
Gpi^{cc}	1	0.68	1	0.11	$Est\text{-}1^{dd}$	–	–	–	0.01
Gpi^{cd}	12	12.51	5	5.45	Lap^{aa}	17	18.92	45	45.79
Gpi^{ce}	1	1.52	–	0.26	Lap^{ab}	51	49.16	49	47.75
Gpi^{cf}	–	0.32	–	0.06	Lap^{ac}	–	–	1	0.66
Gpi^{dd}	59	57.39	65	65.50	Lap^{bb}	31	31.92	12	12.45
Gpi^{de}	10	13.98	7	6.20	Lap^{bc}	–		–	0.34
Gpi^{df}	3	2.95	2	1.56	Lap^{cc}	–	–	–	0.01
Gpi^{ee}	3	0.85	–	0.15					

The enzymatic systems tested are known for their high degree of polymorphism in calanids. O, number of observed specimens; E, frequency expected.

Table II: Allele frequency, observed heterozygosity (H) and degree of agreement between frequency and Hardy–Weinberg expectations (χ^2, P) in the two subsamples analysed

Allele	Subsample I	Subsample II
Gpi^a	–	0.0505
Gpi^b	0.0755	0.0963
Gpi^c	0.0802	0.0321
Gpi^d	0.7358	0.7752
Gpi^e	0.0896	0.0367
Gpi^f	0.0189	0.0092
H	0.3962	0.3670
χ^2; (*P*)	1.5885; (0.82)	0.4917; (0.99)
Est-1^a	0.0044	0.0048
Est-1^b	0.0221	0.1010
Est-1^c	0.9735	0.8846
Est-1^d	–	0.0096
H	0.0354	0.1923
Lap^a	0.4350	0.6542
Lap^b	0.5650	0.3411
Lap^c	–	0.0047
H	0.51	0.4673
χ^2; (*P*)	0.1410; (0.71)	0.0618; (0.82)

product of this allele, which is very characteristic in appearance, made up >10% of the genetically interpreted specimens in subsample II, whereas not a single such specimen was found in subsample I. The statistically significant differences between the subsamples in the allele frequencies of all the loci tested must also be considered highly characteristic. Thus, the two subsamples were both quantitatively and qualitatively different.

Two inferences can be drawn from these results. First, individuals of *C. carinatus* arrive with the upwelling waters on the shelf off Ghana in two genetically different stocks. Secondly, the marker of the genetic subdivision of the *C. carinatus* population could be the gene *Gpi*^{a}. This result is indicative of the non-random (geographic, or perhaps behavioural) segregation of the *C. carinatus* population in the SACW off the Ghanaian coast. Further studies on the differentiation of *C. carinatus* employing markers at the DNA level, based on this investigation, could provide relevant information against the background of debate on the paths of speciation in oceanic plankton [reviewed in e.g. (Jackson and Cheetham, 1999)].

REFERENCES

Adam, D. (1987) Allozyme variation in rye, *Secale cereale* L. *Theor. Appl. Genet.*, **74**, 560–565.

Arashkevich, E. G. and Drits, A. V. (1997) Ekologo-fiziologicheskie pokazateli u *Calanoides carinatus* (Kroyer) Bengal'skogo apvellinga. *Okeanologiya*, **37**, 571–577.

Arashkevich, E. G., Drits, A. V. and Timonin, A. G. (1996) Diapause in the life cycle of *Calanoides carinatus* (Kroyer) (Copepoda, Calanoida). *Hydrobiologia*, **320**, 197–208.

Bakun, A. (1978) Guinea current upwelling. *Nature*, **271**, 147–150.

Hebert, P. D. N. and Beaton, M. J. (1989) *Methodologies for Alloenzyme Analysis Using Cellulose Acetate Electrophoresis*. Helena Laboratory, Beaumont, TX.

Jackson, J. B. C. and Cheetham, A. H. (1999) Tempo and mode of speciation in the sea. *Trends Ecol. Evol.*, **14**, 72–77.

John, H. C., Mittelstaedt, E. and Schulz, K. (1998) The boundary circulation along the European continental slope as transport vehicle for two calanid copepods in the Bay of Biscay. *Oceanol. Acta*, **21**, 307–318.

Kosobokova, K. N., Drits, A. V. and Krylov, P. I. (1988) Fiziologicheskie i biokhimicheskie kharakteristiki *Calanoides carinatus* v vodakh apvellinga u beregov Namibii. *Okeanologiya*, **28**, 484–489.

Kuipers, B. R., Witte, H. J. and Gonzalez, S. R. (1993) Zooplankton distribution in the coastal upwelling system along the Banc d'Arguin, Mauritania. *Hydrobiologia*, **258**, 133–149.

Mensah, M. A. (1974) The reproduction and feeding of the marine copepod *Calanoides carinatus* (Krøyer) in Ghanaian waters. *Ghana J. Sci.*, **14**, 167–191.

Parker, P. G., Snow, A. A., Schug, M. D., Booton, G. C. and Fuerst, P. A. (1998) What molecules can tell us about populations: choosing and using a molecular marker. *Ecology*, **79**, 361–382.

Peterson, W. T. and Painting, S. J. (1990) Developmental rates of the copepods *Calanus australis* and *Calanoides carinatus* in the laboratory, with discussion of methods used for calculation of development time. *J. Plankton Res.*, **12**, 283–293.

Postel, L., Arndt, E. A. and Brenning, U. (1995) Rostock zooplankton studies off West Africa. *Helgol. Meeres.*, **49**, 829–847.

Sunnucks, P. (2000) Efficient genetic markers for population biology. *Trends Ecol. Evol.*, **15**, 199–203.

Sywula, T., Głażewska, I., Koszteyn, J., Kwaśniewski, S. and Sell, J. (1993) An analysis of the population structure of *Calanus* cf. *finmarchicus* (Copepoda) from the Hornsund Fiord region, Spitsbergen. *Variab. Evol.*, **2/3**, 113–119.

Timonin, A. G., Arashkevich, E. G., Drits, A. V. and Semenova, T. N. (1992) Zooplankton dynamics in the northern Benguela ecosystem, with special reference to the copepod *Calanoides carinatus*. *S. Afr. J. Mar. Sci.*, **12**, 545–560.

Valentin, J. L., Monteiro-Ribas, W. M., Mureb, M. A. and Pessotti, E. (1987) On some zooplankters common in the Cabo Frio (Brazil) upwelling. *J. Plankton Res.*, **9**, 1195–1216.

Williams, R. and Conway, D. V. P. (1988) Vertical distribution and seasonal numerical abundance of the Calanidae in oceanic waters to the south-west of the British Isles. *Hydrobiologia*, **167–168**, 259–266.

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