Animal Genetics
Volume 37 Page 348 - August 2006
http://dx.doi.org/10.1111/j.1365-2052.2006.01481.x

The definitive version is available at www.blackwell-synergy.com

An amylase gene polymorphism is associated with growth differences in the Pacific cupped oyster *Crassostrea gigas*

M Prudence[1], J Moal[1], P Boudry[2], JY Daniel[1], C Quéré[1], F Jeffroy[1], C Mingant[1], M Ropert[3], E Bédier[4], A Van Wormhoudt[5], JF Samain[1] and A Huvet[1]*

[1]UMR100 PE2M, Ifremer Centre de Brest, 29280 Plouzané, France;
[2]LGP Ifremer, 17390 La Tremblade, France;
[3]LERN Ifremer, 14520 Port en Bessin, France;
[4]LERMPL Ifremer, 56470 La Trinité, France;
[5]UMR5178 MNHN, 29900 Concarneau, France.

*: Corresponding author : Arnaud.Huvet@ifremer.fr; Phone: 33 2 98 22 46 93 Fax: 33 2 98 22 46 53

Abstract:

This study investigated the non-neutrality of genetic polymorphism in two alpha-amylase genes (AMYA and AMYB) in the oyster Crassostrea gigas. Bi-parental oyster families, bred to be polymorphic for markers in these genes, were monitored for growth and survival for 1 year under standard culture conditions in two French production sites. Within-family genotype frequencies indicated that the two amylase genes were closely linked (c. 1.7 cM). Within two of three families, significant differences in growth were observed between genotypes at one of the two production sites, suggesting that this polymorphism is not neutral and might be under selection because of its role in digestive function. Estimated daily yields were different between amylase genotypes, indicating the potential value of amylase markers in selective breeding programmes to improve oyster growth.

Introduction

Numerous examples of the non-neutrality of *amylase* polymorphisms, a key enzyme for starch digestion, exist in several animal species. For example in chicken, an a*mylase* polymorphism was reported to affect growth and food conversion (Hughes *et al.* 1994).

In marine bivalves, activities of digestive enzymes, especially alpha-amylase, have been reported to control assimilation rate which is one of the principal factors explaining growth variation. In the Pacific oyster *C. gigas*, genes (*AMYA* and *AMYB*) coding for two *amylase* mRNAs have been characterised. Polymorphism in these genes has been analysed by PCR-RFLP (Sellos *et al*. 2003). Analyses of polymorphism at a microsatellite locus present in intron 4 of *AMYA* lead to the hypothesis that selective processes were at work at this microsatellite locus in a *Crassostrea angulata* population (Huvet *et al*. 2004).

In the present study, non-neutrality of *alpha-amylase* polymorphisms in oyster was tested by examining relationships between *amylase* genotype and growth.

Material and methods

Wild oysters, heterozygote for two *amylase* markers, were used as parents to produce five bi-parental families (Table 1). The families, consisting of animals of homogeneous size, were placed under standard culture (450 oysters per bag, 3 bags per family) at 9 months in two rearing sites: Fort-Espagnol in southern Brittany and Baie des Veys sound in Normandy, for one year (March 2003 orT0 to February 2004 or T1). At T0 and T1 (families 2, 3 and 5 were sampled at T1), approximately 150 oysters were randomly collected from each family for genotyping using PCR-RFLP as described in Sellos *et al*. (2003) and weighted (total wet weight and wet flesh weight). Mortality was estimated at T1.
A novel PCR-RFLP allele in gene *B* (*AMYB6*) was identified (Genbank accession number n° DQ286955).

At T1, amylase activity (Le Moine *et al*. 1997) and apparent Michaelis-Menten constant (K_M) were carried out on three replicate pools of ten digestive glands of each genotype collected from families 2 and 3 at Fort-Espagnol.

All statistical analysis, including tests of normality, were performed using one-way analyses of variance followed by multiple comparison tests with the Tukey's HSD method using STATGRAPHICS software. Survival was analysed after angular transformation. Comparisons of genotypic distributions were made using Chi-square tests.

Results and discussion

Observed genotypes and their frequencies at T0 are highly consistent with those expected under the hypothesis of a strong linkage between genes *AMYA* and *AMYB* (Table 1). Different *amylase* genes have been reported on the same chromosome in many organisms (Gumucio *et al.* 1988) and are separated by a maximum of 0.87 cM in mouse (Bloor & Meisler 1980). Our results suggest a mean genetic distance of 1.7 cM between the two *amylase* genes in *C. gigas.*

Significant within families differences between genotypes in total and flesh weights were observed in Brittany at T1 in families 2 and 3 (Figure 1). In family 2, two significant groups were observed: *AMYA1/2,AMYB2/2*, which had the highest total and flesh weights; *AMYA1/5,AMYB1/6*, which had significantly lower weights than *AMYA1/2,AMYB2/2*; and finally *AMYA1/1,AMYB1/2* and *AMYA2/5,AMYB2/6* which showed intermediate values. Within family 3, the weights of the four genotypes also clustered into two significantly different groups (Figure 1). Differences between the extreme genotypes within family 2 (*AMYA1/2,AMYB2/2* and *AMYA1/5,AMYB1/6*) were 4.9g and 0.5g for total and flesh weights respectively, corresponding to differences of 32% and 37%. Taking into account differences in weight and similarity of survival (Table 1), estimated daily yield ranged from 3% per day

for genotype *AMYA1/5,AMYB1/6* to 3.7% per day for genotype *AMYA1/2,AMYB2/2* maintained at Fort-Espagnol.

Weight differences between *amylase* genotypes were only apparent in the Brittany site. It should be noted that food availability was lower in Brittany than in Normandy (mean chlorophyll a levels in Fort-Espagnol, Brittany = 3.3 µg/L and in Baie des Veys, Normandy = 4.9 µg/L). This genotype x environment interaction suggests that certain *amylase* genotypes might be better adapted to certain environments than others.

Given that genotypes present in families 2 and 3 showed significant differences in total and flesh weights in Brittany, amylase enzymatic parameters were analysed. Differences of amylase activity among genotypes approached significance in family 3 (P= 0.09). Interestingly, *AMYA3/6,AMYB1/1* showed both the highest weights and highest values of specific amylase activity (1.38±0.23 IU/mg soluble protein) and *AMYA1/6,AMYB1/5* had the lowest values of both parameters (activity= 0.94±0.04 IU/mg). Growth differences between genotypes in this family might be partly explained by differences in specific amylase activity but further exploration is needed to determine differences in enzyme quantity, as found in *Drosophila melanogaster* (Hickey 1981) and chicken (Hughes *et al*. 1994).

In conclusion, the observed differences in growth between *amylase* genotypes in *C. gigas* suggests that these polymorphisms are not neutral and could be under selection due to their digestive function, as previously suggested in chickens (Hughes *et al*. 1994). This finding is also supported by the putative non-neutrality of a microsatellite in *AMYA*, that is in linkage disequilibrium with *amylase* exons in a Portuguese population of oysters (Huvet *et al*. 2004). Alternatively, *amylase* genes might be linked to other non-neutral genes (i.e. genetic hitchhiking; Barton, 2000). Development of linkage maps, identification of QTLs and genome characterization of *C. gigas* (Hedgecock *et al*. 2005) will advance these studies. Further investigations of digestive (absorption, assimilation) and amylase parameters are needed.

Acknowledgements

We are indebted to H. McCombie for her help with the English. We thank L. Dégremont and the staff of the Argenton, Bouin, La Trinité and Port en Bessin stations for the maintenance of oysters. M. Prudence was funded by a Région Basse-Normandie doctoral grant. This work was partly supported by the Bureau des Ressources Génétiques (contract n°05/5210460/YF).

References

Barton N.H. (2000) Genetic hitchhiking. *Philosophical Transactions of the Royal Society of London. Series B, Biological sciences* **355**, 1553-62.

Bloor J.H. & Meisler M.H. (1980) Additional evidence for the close linkage of *amy1* and *amy2* in the mouse. *The Journal of heredity* **71**, 449-51.

Gumucio D.L., Wiebauer K., Caldwell R.M., Samuelson L.C. & Meisle, M.H. (1988) Concerted evolution of human *amylase* genes. *Molecular and Cellular Biology* **8**, 1197-205.

Hedgecock D., Gaffney P.M., Goulletquer P., Guo X.M., Reece K. & Warr G.W. (2005) The case for sequencing the Pacific oyster genome. *Journal of Shellfish Research* **24**, 429-41.

Hickey D.A. (1981) Regulation of amylase activity in *Drosophila melanogaster*: variation in the number of enzyme molecules produced by different amylase genotypes. *Biochemical Genetics* **19**, 783-96.

Hughes B.L., Suniga R.G. & Yardley D.G. (1994) Influence of *amylase* genotypes on growth rate and feed conversion of chickens. *Poultry Sciences* **73**, 953-7.

Huvet A., Fabioux C., McCombie H., Lapègue S. & Boudry P. (2004) Natural hybridization between genetically differentiated populations of *Crassostrea gigas* and *C. angulata* highlighted by sequence variation in flanking regions of a microsatellite locus. *Marine Ecology Progress Series* **272**, 141-52.

Le Moine S., Sellos D., Moal J., Daniel J.Y., Serrano F.S.J., Samain J.F. & Van Wormhoudt A. (1997) Amylase in *Pecten maximus*: protein and cDNA characterization, quantification of the expression in the digestive gland. *Molecular Marine Biology and Biotechnology* **6**, 228-37.

Sellos D., Moal J., Dégremont L., Huvet A., Daniel J.Y., Nicoulaud S., Boudry P., Samain J.F. & Van Wormhoudt A (2003) Structure of *amylase* genes in populations of Pacific cupped oyster (*Crassostrea gigas*): tissue expression and allelic polymorphism. *Marine Biotechnology* **5**, 360-72.

Table 1 Expected and observed genotype frequencies for polymorphisms in two *amylase* genes (*AMYA*, *AMYB*) at the beginning of the experiment (T0) and in families 2, 3 and 5 at the end of the experiment (T1) after one year of rearing in two areas (southern Brittany and Normandy).

Family		Parental genotypes	Progeny genotypes	Expected frequency[1]	Observed frequency T0 (number of individuals)	Observed frequency T1 Brittany (number of individuals)	Observed frequency T1 Normandy (number of individuals)
1			*AMYA1/1,AMYB5/5*	0.25	0.25 (44)		
	Dam	*AMYA1/2,AMYB1/5*	*AMYA1/2,AMY/1B5*	0.50	0.48 (84)		
	Sire	*AMYA1/2,AMYB1/5*	*AMYA2/2,AMYB1/1*	0.25	0.26 (46)		
			AMYA1/1,AMYB1/5	0	0.005 (1)		
			AMYA1/2,AMYB1/1	0	0.005 (1)		
2			*AMYA1/1,AMYB1/2*	0.25	0.19 (26)	0.23 (34)	0.24 (36)
			AMYA1/2,AMYB2/2	0.25	0.24 (32)	0.22 (33)	0.32 (48)
	Dam	*AMYA1/2,AMYB1/2*	*AMYA1/5,AMYB1/6*	0.25	0.23 (30)	0.34 (50)	0.22 (33)
	Sire	*AMYA1/5,AMYB2/6*	*AMYA2/5,AMYB2/6*	0.25	0.3 (40)	0.21 (31)	0.22 (32)
			AMYA1/5,AMYB2/6	0	0.02 (3)		
			AMYA2/5,AMYB2/2	0	0.02 (2)		
3			*AMYA1/2,AMYB2/5*	0.25	0.26 (33)	0.28 (42)	0.28 (42)
	Dam	*AMYA1/3,AMYB1/5*	*AMYA1/6,AMYB1/5*	0.25	0.25 (31)	0.27 (40)	0.26 (39)
	Sire	*AMYA2/6,AMYB1/2*	*AMYA2/3,AMYB1/2*	0.25	0.19 (23)	0.18 (27)	0.23 (35)
			AMYA3/6,AMYB1/1	0.25	0.30 (38)	0.27 (41)	0.23 (34)
4			*AMYA1/1,AMYB1/5*	0.25	0.25 (33)		
	Dam	*AMYA1/3,AMYB1/5*	*AMYA1/3,AMYB1/1*	0.25	0.28 (37)		
	Sire	*AMYA1/3,AMYB1/3*	*AMYA1/3,AMYB3/5*	0.25	0.22 (30)		
			AMYA3/3,AMYB1/3	0.25	0.25 (34)		
5			*AMYA1/2,AMYB2/3*	0.25	0.19 (24)	0.29 (43)	0.19 (29)
			AMYA2/2,AMYB1/2	0.25	0.23 (30)	0.14 (21)	0.21 (31)
	Dam	*AMYA2/6,AMYB1/2*	*AMYA1/6,AMYB1/3*	0.25	0.22 (29)	0.29 (43)	0.33 (50)
	Sire	*AMYA1/2,AMYB1/3*	*AMYA2/6,AMYB1/1*	0.25	0.32 (41)	0.28 (42)	0.27 (40)
			AMYA1/2,AMYB1/2	0	0.01 (1)		
			AMYA1/2,AMYB1/3	0	0.01 (1)		
			AMYA2/2,AMYB1/1	0	0.02 (3)		

[1] expected genotype frequencies are given under the hypothesis of linkage between *amylase* genes.

Figure 1 Total weight and flesh wet weight in grams (g) across genotypes in two *amylase* genes (*AMYA,AMYB*) in two oyster families (Fig. 1A: family 2; Fig. 1B: family 3) after one year of rearing in two areas (black bar for southern Brittany and grey bar for Normandy). Homogenous groups were estimated between genotypes within family in southern Brittany using multiple comparison tests according to the Tukey's HSD method.

Fig. 1A

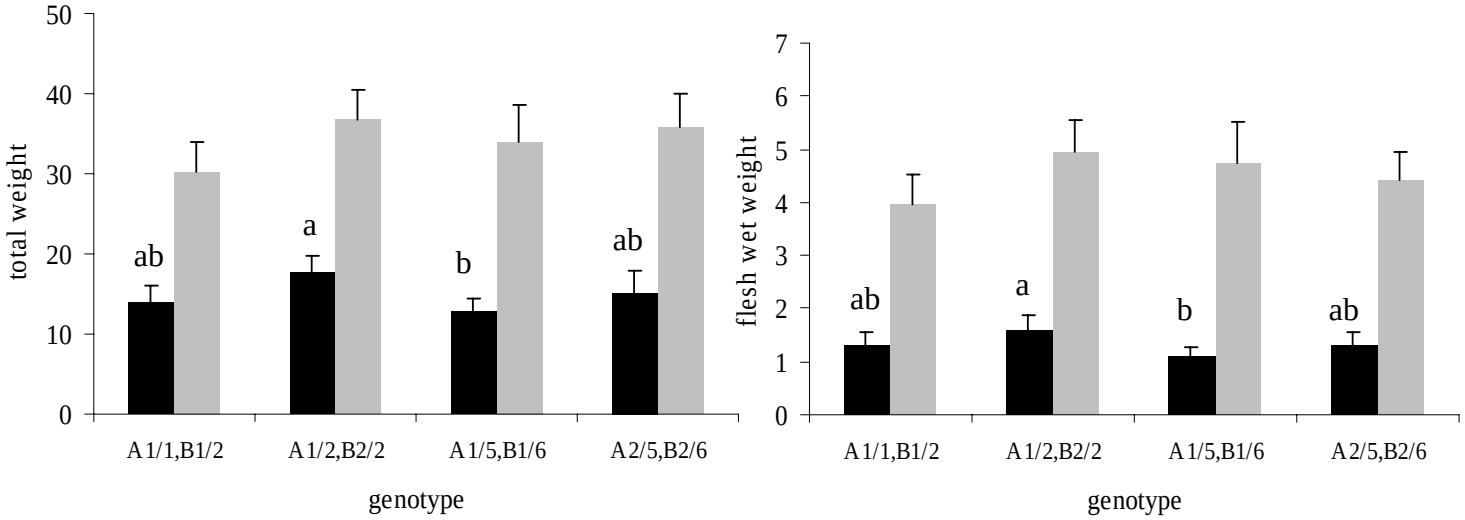

Fig. 1B