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INHERITANCE OF PREDOMINANTLY HIDDEN SHELL COLOURS IN *MACOMA BALTHICA* (L.) (BIVALVIA: TELLINIDAE)

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ABSTRACT

Adults of the bivalve *Macoma balthica* live burrowed in mud or sand, which makes their shells invisible so that a vision-related selective advantage of colour morphs is hard to imagine. Nevertheless, this species harbours a conspicuous colour polymorphism of white, yellow, orange and red. Here we present data from laboratory-reared crosses that demonstrate a genetic basis for this colour polymorphism. The results suggest a simple model of inheritance of colour class: four alleles at a single locus displaying linear hierarchy of dominance, in the following order of increasing dominance: white < yellow < orange < red. Shell colour intensity is also shown to be a heritable trait in *M. balthica*. Furthermore, bicolouration may to a degree be a heritable trait, but the sample size is too small to draw any conclusions about possible inheritance mechanisms. Heritable colour variation can now, with the proposed model, be used as a genetic marker. Furthermore, the proposed model will provide the basis for further analysis of the evolutionary forces maintaining this colour polymorphism that is hidden from view for most of the life of this bivalve.

INTRODUCTION

The widespread colour polymorphisms of molluscan shells have fascinated humans throughout historical times. Conspicuously coloured shells were used as currency in many cultures (Monnerie, 2002; Saul, 2004), and in some places they still are, e.g. on some of the Pacific islands. Biologists are intrigued by colour polymorphisms because it combines several phenomena – biochemistry, inheritance, genetic drift, directional selection and frequency-dependent selection (Jones, Leith & Rawlings, 1977; Hedegaard, Bardeau & Chateigner, 2006). It might seem obvious to associate shell colour polymorphisms primarily with selective advantages related to vision. Yet, it is important to note that evidence from the fossil record suggests that shell colour patterns evolved earlier than the vision sufficiently sophisticated to see them (Kobluk & Mapes, 1989).

Among the temperate and boreal marine bivalves of the northern hemisphere, arguably the most brilliantly coloured shells are produced by members of the Tellinoidea (Cain, 1988), which spend most of their lives burrowed in the sediment, and therefore not visible at all. Moreover, in several of the Tellinoidea, colour is predominantly present on the inside of the shells, the outside being whitish, a disparity which increases with age. Studying such colour polymorphisms that are hidden from view will contribute to our understanding of colour polymorphisms in general. This paper presents the first analysis of the genetic basis of a shell colour polymorphism in the Tellinoidea, namely in the Baltic tellin *Macoma balthica* (L.), which occurs in bright colours ranging from white to yellow, orange and red and is hidden from view for most of its life history.

Macoma balthica is sedentary throughout its adult life and burrows in sand or mud down to about 8 cm deep (Zwarts *et al.*, 1994; Goeij & Luttikhuizen, 1998; Goeij, 2001). Its separate sexes reproduce in early spring through external fertilization (Honkoop, Luttikhuizen & Piersma, 1999). The colourless larvae lead a pelagic, planktotrophic life for three to five weeks (Caddy, 1967; Drent, 2002), after which they metamorphose and settle mostly high in the intertidal (Beukema, 1993). In the winter of their first year, a high proportion of the juveniles migrate by drifting with the use of a mucous thread (Sörlin, 1988; Beukema & De Vlas, 1989; Hiddink, Kock & Wolff, 2002; Beukema & Dekker, 2003). By this time the juveniles are still small, approximately 2 to 10 mm long, and mostly translucent, but the colour of their shells is visible in some, particularly the more brightly coloured ones (Hiddink, 2002). As the animals grow, the colour of the outside and inside of the shells starts to diverge; in most older animals, the inside of the shell continues to display bright colours, while the outside increasingly becomes chalky white.

Both heritable factors and environmental effects have been reported to control molluscan shell colour variation. Most studies report a relatively simple genetic basis for colour polymorphisms involving one or two loci with dominance (Innes & Haley, 1977; Palmer, 1985; Ekendahl & Johannesson, 1997; Yusa, 2004) and more elaborate polymorphisms may be formed by more complicated genetic systems (Peignon *et al.*, 1995; Winkler *et al.*, 2001). Underwood & Creese (1976) and Creese & Underwood (1976), on the other hand, showed that environmental variation in chlorophyll concentration in the substratum is likely to cause variation in banding pattern intensity in the snail *Austrocochlea constricta*.

The question addressed in the present paper is whether the colour variation has a genetic basis or is produced by induction from some factor in the environment (first null hypothesis: colour variation in *M. balthica* has no genetic basis). In the case of a genetic basis, a model of inheritance proposed for this species by Strelkov, Gantsevich & Basova (2001) forms the second null hypothesis: a single-locus model with three alleles 0 (no colour), Y (yellow) and R (red), where the 0-allele would produce no colouring and therefore be recessive; both colour alleles would be codominant and, logical from a colour mixing perspective, heterozygotes YR would be orange. We examined laboratory reared families and compared colouration between offspring and parents.

MATERIAL AND METHODS

Fifty-three families were reared in a common laboratory environment. Briefly, rearing involved the following. The

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parents were induced to spawn by giving them a temperature shock (putting them in water of 15°C after at least 24 h at 5°C). The temperature was held constant at 15°C throughout the remainder of the process. Gametes were combined in glass beakers for fertilization and left overnight. After rinsing, the fertilized unhatched eggs were transferred to mildly aerated columns containing filtered sea water and antibiotics. During the first 3 days in the columns, the eggs hatched. From the fourth day onwards, freshly cultured algae were fed to the larvae. When the larvae showed competence to settle (as judged from the development of a foot), they were transferred to horizontal mesh surfaces suspended in aquaria. When the settled larvae had grown >0.5 mm, they were transferred to aquaria containing incinerated sediment and left to burrow themselves. Aquaria, a separate one for each family, contained aerated sea water which was refreshed every other day. For more details on rearing procedures see Luttikhuizen *et al.* (2003a) and Drent, Luttikhuizen & Piersma (2004).

The parents were collected at four localities: Balsfjord (Norway), North Sea near Terschelling (The Netherlands), Mok (Wadden Sea, The Netherlands) and Gironde (France) (Table 1). The parents used were randomly taken from the populations, and crosses made were also produced randomly with respect to colour. Crosses were made primarily within origin, with the exception of families 25 and 97 that had one Gironde and one Balsfjord parent (Table 1). Several families shared a parent, with a total of 63 parents involved. After the parents had spawned and their gametes used for fertilization, they were opened, their soft body parts removed and their shells air-dried and stored in the dark. A total of 332 offspring shells were sacrificed in their second year and also their soft body parts were removed and their shells air-dried and stored in the dark. Parental and offspring shells were put inside-down on a digital image scanner (Hewlett Packard, Palo Alto, USA) and the images scanned and saved. Of each shell, the colour was measured in Adobe Photoshop 7.0 at three different positions on the inside of the shell, using the HSB (Hue, Saturation, Blackness) colour system. The three different positions were chosen randomly in principle, but avoiding areas that were obviously not displaying the actual hue on the scanned image because of reflection (resulting from the scanning process) or shell damage. Saturation measurements were log-transformed to homogenize the variance. The term 'intensity' is used throughout the remainder of this paper to indicate log-transformed saturation. The origin of hue (a scale of 0–360) was adjusted by subtraction of 276 to fall in between red and white. The three hue and intensity measurements were averaged per shell.

Some of the shells of *Macoma balthica* are bicoloured (see e.g. Cain, 1988), the peripheral part having a different hue than the part closest to the umbones. For consistency, the primary colour was defined as the colour on the peripheral parts of the shell, both in adults and in juveniles, while the colour of the inner ring (called secondary colour here) was also recorded.

Inheritance of shell colour intensity (of primary shell colour) was analysed statistically by sibling and by parent–offspring analyses. First, raw intensity measurements were corrected for an overall difference in intensity between colour classes (white, red, yellow and orange) by taking the residuals of an analysis of variance (ANOVA) with colour class as fixed independent factor. Next, corrected intensities were subjected to a sibling analysis which consisted of an ANOVA (offspring only) with random independent factor 'family'. This test could determine whether corrected intensity varied less within families than between families, indicating a genetic effect. Parent–offspring analyses were carried out to estimate narrow-sense heritability, by linear regression analysis of average offspring corrected intensity against midparent corrected intensity. The slope of the regression was taken as narrow-sense heritability h^2. Finally, it was tested whether colour class corrected intensity inherits independently from colour class, by comparing offspring shell colour with either their mother's or their father's; two general linear models (GLM) were used on the offspring intensity data with the three factors mother/father intensity, colour identical to mother/father or not and the interaction between them both. In the case of independent inheritance of intensity and colour class, the latter two factors were expected to be non-significant.

Table 1. Origin of field collected *Macoma balthica* used as parents for rearing.

Location	Water body	Country	Latitude (N)	Longitude (E)
Bonne Anse	Gironde	France	45°41.46′	−1°12.45′
Mok	Wadden Sea	The Netherlands	53°00.50′	4°45.80′
Terschelling	North Sea	The Netherlands	53°27.00′	5°28.00′
Nordkjosbotn	Balsfjord	Norway	69°13.08′	19°31.35′

RESULTS

Inheritance of primary shell colour

By eye, four hue classes seemed to be present among *Macoma balthica* shells (Fig. 1), and the scanned colour measurements corroborated this approximation (Fig. 2). The hue classes distinguished were red ($0 \leq$ hue < 92), orange ($92 \leq$ hue < 127), yellow ($127 \leq$ hue < 181) and white ($181 \leq$ hue ≤ 360 as well as all values for hue with intensity <2) and each hue came in a continuous range of intensities. It was not always possible to distinguish low-intensity colours from white, neither by eye nor on the basis of scanned data (Fig. 2). Because of this, the colour class data contain a certain degree of noise.

All four hue classes were found among both juveniles and adults, with white being the most frequent hue (52% of juveniles and 49% of adults), followed by red (23% and 21%, respectively), then yellow (19% and 25%, respectively) and orange being the rarer class (6% and 5%, respectively).

Families with two white-shelled parents almost exclusively gave rise to white-shelled offspring, but 9 out of 104 of their offspring, distributed over 5 out of 13 families, were yellow (Table 2). These yellow shells appear to occur only in mixed progenies of white and yellow siblings. Although these data might be taken to suggest that white may be dominant over yellow, the three families of two yellow parents gave rise to yellow and white offspring in similar frequencies and to a single orange one, meaning that the six parents involved cannot all have been homozygote recessives. It is more likely to assume that some of the parents scored as white-shelled were actually a light shade of yellow, which is possible because of the overlap between the two colours in the hue/intensity plot (Fig. 2), and that a putative white allele is recessive to all other putative alleles. Looking at the data in more detail, this is indeed likely: families 2, 58 and 83 had a mother whose shell colour lay in the low-intensity zone of overlap between yellow and white, and families 141 and 297 shared a father with this same characteristic. The nine yellow offspring involved were all a clear yellow.

A model of white being recessive to all other putative alleles is also in accordance with the appearance of white offspring among progenies of two red parents (6 families), which produced mainly red (26) and a few white (4), yellow (6) and orange (3) offspring. Crosses between two yellow parents resulted in only yellow offspring in one case (family 235) and

Figure 1. Shell colour polymorphism in *Macoma balthica* (Bivalvia: Tellinidae). From top to bottom: rows of five each of white, yellow, orange, bicoloured and red shells. Shown is inside of shells, in a range of intensities. Sample shown was collected at Eemshaven, Wadden Sea (53°28′N, 6°48′E, 1 May 2007) and is a selection from a total sample size of 250 individuals. Scale is actual size.

equal numbers of white and yellow offspring in another (family 160). Family 203, which consisted of two parents with yellow as its primary colour and a single orange offspring, is treated later because the mother was a bicoloured individual with orange as secondary colour. In the random design, crossings between two orange parents did not occur.

Crosses between parents of different hues involving one white parent yielded different results for the three colour classes. A white and a yellow parent almost always gave rise to yellow shells among their progeny (9 out of 11 families) and frequently to white shells (7 out of 11 families). They would never segregate red or orange. A white and a red parent would almost always produce red (eight out of nine families) as well as white (seven out of nine families) shells among their offspring. In addition, this combination was found to segregate yellow shells, while no orange shells were observed among the offspring. Finally, a white and an orange parent always resulted in white and frequently in orange shells among the offspring (four out of six families). Segregation of yellow was also observed among these white-by-orange families, represented by a single offspring among two white and two orange siblings (family 206, see Table 2).

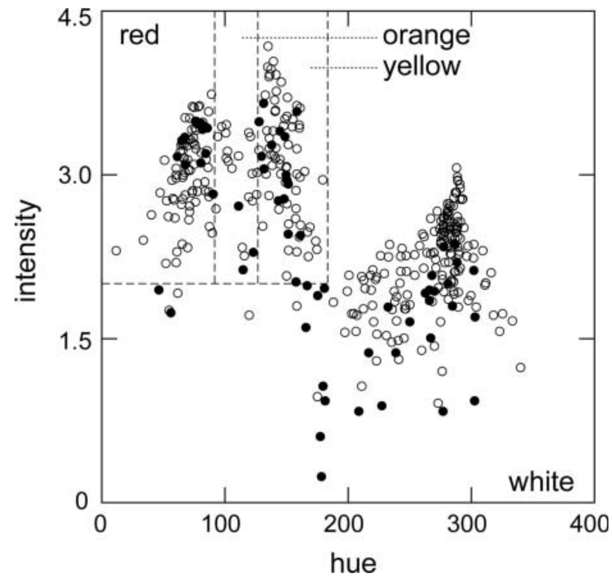

Figure 2. Colour polymorphism among *Macoma balthica* parents ($n = 63$) and offspring ($n = 332$), characterized on the basis of hue and intensity. Each point is the average over three measurements per shell, performed on inner shell surface. Open symbols are offspring, filled symbols are parents.

Crosses between two non-white parents were only carried out for yellow and red parents due to the rarity of the orange morph. Among their progeny were almost always white (four out of five families) and/or red (also four out of five families) shells, and frequently yellow (three out of five families) and orange (also three out of five families) morphs segregated.

These results reject the first hypothesis that shell colour class in *M. balthica* has no genetic basis. Furthermore, the second null hypothesis that orange shells codominantly express a red and a yellow allele is also rejected; in that case, predominant segregation of yellow and red is expected in white-by-orange crosses. Instead, the results support a model of four putative colour alleles at a single locus, displaying a linear hierarchy of dominance, in the following order of increasing dominance: white < yellow < orange < red.

Inheritance of shell colour intensity

Intensity of shell colour was also found to be under genetic control. This was evidenced by the sibling analysis shown in Table 3. Differences between colour classes in intensity were corrected for by taking the residual from an ANOVA with measured intensity as dependent and colour as independent factor. A subsequent ANOVA with random independent factor 'family' significantly ($P < 0.001$) explained part of the intensity variation among 324 offspring shells in 45 families (eight single-offspring families were left out of the analysis). This means that intensity varied less within sibships than among sibships, i.e. colour intensity is under genetic control.

Measured intensities tended on the whole to be slightly different for adults (parents) than for juveniles (offspring). Examining this in more detail, it was seen that the intensity was lower in adults among the white and yellow colour classes, and higher among red shells (Fig. 2). Possibly transparency, still present in juveniles but no longer in adults, is a cause of this, having measured the shells against a dark background.

The heritability of intensity is further illustrated by the parent–offspring analysis, shown in Table 4 and Figure 3. Colour-corrected intensity of offspring shells was significantly

Table 2. Primary shell colour of parents and offspring of *Macoma balthica*. For details of measurement see Results section.

Family	Mother*	Father*	Offspring			
			White	Yellow	Orange	Red
50	1(white)B	3(white)B	5	–	–	–
84	4(white)B	3(white)B	5	–	–	–
N4	34(white)B	29(white)B	24	–	–	–
N6	34(white)B	31(white)B	5	–	–	–
112	45(white)N	42(white)N	12	–	–	–
209	46(white)N	42(white)N	4	–	–	–
114	55(white)W	58(white)W	10	–	–	–
48	97(white)W	93(white)W	1	–	–	–
2	8(white)G	5(white)G	3	*2*	–	–
141	75(white)N	78(white)N	14	*4*	–	–
58	50(white)W	48(white)W	12	*1*	–	–
83	6(white)G	12(white)G	–	*1*	–	–
297	74(white)N	78(white)N	–	*1*	–	–
235	13(yellow)G	14(yellow)G	–	4	–	–
160	89(yellow)W	88(yellow)W	9	10	–	–
203	66(yellow)N	61(yellow)N	–	–	*1*	–
N8	33(red)B	30(red)B	–	–	–	2
54	63(red)N	64(red)N	–	–	–	1
223	65(red)N	64(red)N	–	–	1	4
N2	32(red)B	30(red)B	–	–	1	8
159	65(red)N	62(red)N	–	6	*1*	5
144	63(red)N	62(red)N	4	–	–	6
70	13(yellow)G	12(white)G	–	3	–	–
36	15(yellow)G	12(white)G	–	5	–	–
56	49(yellow)W	48(white)W	–	1	–	–
11	92(yellow)W	95(white)W	–	3	–	–
137	49(yellow)W	47(white)W	1	–	–	–
71	51(yellow)W	48(white)W	5	–	–	–
197	13(yellow)G	16(white)G	3	2	–	–
75	22(yellow)G	27(white)G	2	3	–	–
222	56(yellow)W	59(white)W	2	2	–	–
280	24(white)G	18(yellow)G	1	4	–	–
238	8(white)G	9(yellow)G	3	2	–	–
N1	32(red)B	29(white)B	3	–	–	2
N7	33(red)B	29(white)B	1	–	–	1
69	84(red)W	82(white)W	1	–	–	1
100	26(white)G	17(red)G	2	–	–	3
236	50(white)W	52(red)W	2	–	–	3
N5	34(white)B	30(red)B	–	–	–	21
N9	33(red)B	31(white)B	–	–	–	1
279a	45(white)N	44(red)N	6	*4*	–	–
212	77(red)N	78(white)N	1	*1*	–	2
97	8(white)G	7(orange)B	4	–	1	–
102	43(white)N	41(orange)N	4	–	5	–
25	1(white)B	2(orange)G	5	–	–	–
174	4(white)B	2(orange)G	5	–	–	–
127	46(white)N	41(orange)N	2	–	3	–
206	45(white)N	41(orange)N	2	*1*	2	–
259	83(yellow)W	80(red)W	1	2	2	–
109	66(yellow)N	64(red)N	–	–	1	1
183	49(yellow)W	52(red)W	2	–	*1*	2
192	66(yellow)N	62(red)N	3	1	–	6
118	77(red)N	73(yellow)N	5	1	*1*	5

*Parental codes consist of a number designating individual, followed by colour (in brackets) and a letter indicating location of origin (G, Gironde; W, Wadden Sea; N, North Sea; B, Balsfjord; see Table 1). The values in italics are offspring colours unexpected on the basis of proposed inheritance model; they are discussed in the text.

Table 3. Sibling analysis by ANOVA of inheritance of level of colour intensity in *Macoma balthica*. Independent variable is intensity corrected for colour class (white, red, yellow or orange).

Source	df	Mean-square	*F*-ratio	*P* value
Intercept	1	1.297	10.112	<0.01
Family	44	0.576	4.486	<0.001
Error	279	0.128		

related to midparent colour-corrected intensity (linear regression, $P < 0.01$, $r^2 = 16.9\%$). Narrow-sense heritability for intensity was estimated as the slope of the regression: $h^2 = 0.314$ (standard error 0.097).

More detailed parent–offspring analyses suggest that intensity is inherited independently from colour, i.e. parental colour intensity also influences offspring colour intensity when their shells are not the same colour. Sibships were split up according to whether they expressed the same or a different colour than their parent. Mean corrected intensity of the offspring was found to be significantly influenced by their mother's corrected intensity (GLM, $P < 0.01$, Table 4), and this effect did not depend on whether the offspring differed from the mother in shell colour or not (GLM, n.s., Table 4). The same results were obtained when comparing offspring with their fathers (Table 4).

In summary, shell colour intensity is a heritable trait in *Macoma balthica*, which is transmitted independently from colour itself. This suggests that the loci encoding colour and intensity are unlinked.

Bicoloured shells

Among the 63 parents, six were judged by eye to be bicoloured (9.5%; data on bicoloured shells are shown in Table 5). Two of these were females and four males. After automated colour determination, one of the males was designated non-bicoloured, as both its outer and central shell came out as white from the scanner data.

Among the offspring, 10 out of 332 were scored by eye as bicoloured, amounting to 3.0%, i.e. less than among the adults. One of the offspring was not bicoloured on the basis of scanner data, with both inner and central shell coming out as white (family 209 and individual 4). Bicoloured offspring did not appear to be randomly distributed over the families, as a pair of them occurred in both families 11 (two bicoloured out of three) and 206 (two bicoloured out of five). In addition, two instances of a bicoloured parent–offspring couple were observed: parent 66 is the mother of one bicoloured offspring (out of 13) and parent 14 is the father of one bicoloured offspring (out of four). However, in neither of the latter cases were the parent and offspring similar; all four had a yellow primary colour but parent 14 was white in its centre, while its offspring was orange there, and the reverse was seen for parent 66 and her offspring.

The colours of bicoloured shells did not occur in similar frequencies as were seen among the primary colours of the population as a whole. While white is by far the most frequent primary hue in the population, bicoloured shells most often have yellow as their primary colour (64%) and orange as their secondary colour. Red was not recorded for any of the bicoloured shells.

It is likely from these observations that bicolouration is a to a degree heritable trait, but the sample size is too small to draw any conclusions about possible inheritance mechanisms.

DISCUSSION

The results presented demonstrate a genetic basis for variation in shell colour as well as for variation in colour intensity for *Macoma balthica*. This knowledge is the first step in the process of understanding the maintenance of shell colour variation which is hidden from view – hidden, in the case of *M. balthica*, because the colours are most vivid on the inside of the shells and because the animals are burrowed in sediment for most of their lives. The fact that we are dealing with a genetic polymorphism means that important factors to consider in future studies, in addition to selection factors, are mutation and genetic drift.

Similar to Beukema & Meehan (1985) and Strelkov *et al.* (2001), we discriminate four phenotypic classes: white, yellow, orange and red. Cain (1988), in contrast, discriminated 30 different colour classes in the same hue range, distinguishing light and dark shades as well as several yellows (e.g. 'chrome yellow' and 'lemon yellow'), oranges (e.g. 'deep orange' and 'medium orange') and reds (e.g. 'deep pink' and 'peach'). A simple genetic model of dominance at a single locus is consistent with the data for inheritance of colour class we present, with four allelic variants, which are, in the order of decreasing

Table 4. Parent–offspring analysis of inheritance of colour intensity in *Macoma balthica*. Intensity was corrected for variation among colour classes by ANOVA. CI, shell colour intensity, corrected for colour class; CI_{mid}, midparent CI (average of parents' CIs); CI_{mat}, maternal CI; CI_{pat}, paternal CI; ID, shell colour identical to parent or not; df, degrees of freedom; SE, standard error.

Factor	df	Mean-square	*F*-ratio	*P* value	Coefficient (SE)
Regression, midparent against offspring mean					
CI_{mid}	1	1.019	10.4	<0.01	0.314 (0.097)
Error	51	0.098			
GLM, mother against offspring mean					
CI_{mat}	1	0.551	4.451	<0.05	
ID	1	0.401	3.241	n.s.	
ID × CI_{mat}	1	0.118	0.957	n.s.	
Error	76	0.124			
GLM, father against offspring mean					
CI_{pat}	1	1.154	10.392	<0.01	
ID	1	0.034	0.303	n.s.	
ID × CI_{pat}	1	0.371	3.340	n.s.	
Error	76	0.111			

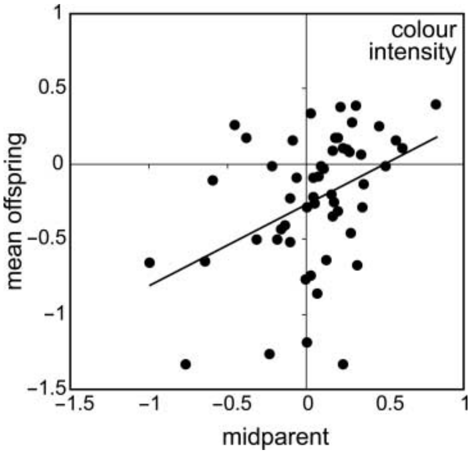

Figure 3. Parent–offspring regression of shell colour intensity in *Macoma balthica* (linear regression, $P < 0.01$, $r^2 = 16.9\%$). Intensity is corrected for shell colour class. Each dot represents mean intensity among offspring within one family, plotted against mean intensity of the parents.

Table 5. Hue and intensity for bicoloured offspring and parents among 53 *Macoma balthica* families.

Individual	Primary colour (outer ring)			Secondary colour (inner ring)		
	Hue	Intensity	Colour class	Hue	Intensity	Colour class
Parents						
4	302.7	2.12	White	129.7	2.78	Yellow
7	111.7	2.71	Orange	204.7	2.85	White
14*	149.3	3.34	Yellow	206.0	3.37	White
16	303.3	0.92	White	121.7	2.88	Orange
31†	176.0	1.89	White	210.0	1.89	White
66‡	130.3	3.16	Yellow	95.7	3.10	Orange
Offspring						
11-2	141.7	3.14	Yellow	106.3	3.23	Orange
11-3	139.0	3.08	Yellow	105.0	3.24	Orange
58-7	145.3	3.09	Yellow	113.3	1.89	White
70-2	136.3	3.89	Yellow	118.7	3.65	Orange
159-5	134.7	3.4	Yellow	108.7	3.34	Orange
192-7‡	131.3	2.52	Yellow	25.7	1.83	White
206-1	116.3	2.25	Orange	140.3	2.32	Yellow
206-3	114.7	2.33	Orange	134.7	2.78	Yellow
209-4†	55.3	1.75	White	306.0	1.93	White
235-2*	135.3	3.98	Yellow	118.0	4.03	Orange

*Male 14 is the father of offspring no. 235-2; †shells appeared bicoloured by eye (primary colour of parent 31 was judged light yellow by eye and that of offspring 209-4 red) but not on the basis of scanned colour class determination; ‡female 66 is the mother of offspring no. 192-7.

dominance: red, orange, yellow and white. A previously suggested model of orange as heterozygote for a red and a yellow allele in *M. balthica* (Strelkov *et al.*, 2001) is thus not corroborated. Colour intensity is also at least partially genetically determined. Because the results suggest that intensity inherits independently from colour class, the loci coding for the two traits are probably not tightly linked. Bicolouration, with the part of the shell closest to the umbones having a different colour than the outer ring, also appears to have a genetic basis, but generalizations cannot be made on the basis of our data.

The frequencies of the colour classes in the general population are rather different from the classes found in bicoloured individuals. Most striking are the rarity of white and red in bicoloured shells. These observations may be related to a partial transparency of the shell material. However, although transparency may explain why red was never recorded for the inner part of the bicoloured shells, it cannot explain why the outer ring was never red, and white only once. If transparency does play a role, the actual frequency of bicoloured shells is expected to be higher than the ∼10% observed among adults. The frequency among our juveniles is almost certainly underestimated and would have increased had the shells been allowed to grow to a larger size.

Given our proposed model of a linear dominance hierarchy of four alleles, a number of offspring colours as depicted in Table 2 can be considered as unexpected. Contamination between families with gametes or juveniles is a possible technical cause of unexpected results, although extreme care was taken to prevent this. However, most of these unexpected results can be either explained by the rigorous subdivision into colour groups, which was apparently faulty for some low-intensity yellow parents, or by the presence of bicoloured individuals among parents and offspring. Adults that should probably, on the basis of their offsprings' colours, be considered as low-intensity yellow are 6, 8, 45 and 78 (Table 2). They had hue scores at the border between yellow and white but with intensities below 2. On the basis of their offspring, they probably carried a 'yellow'-allele. Similarly, family 203 consisted of three high-intensity individuals: a clearly yellow father and a mother and offspring whose hues were at the border between yellow and orange. Unexpected offspring phenotypes related to bicolouration may have occurred in families 58, 118, 159 and 206 (Table 2). These families contained one or two bicoloured offspring (Table 5). Expression of bicolouration may not yet have happened in the smaller offspring; at the same time, bicolouration in the offspring may also indicate that bicolouration in the parents has gone undetected. Since we as yet have no knowledge on the developmental or genotypic nature of the expression of bicolouration in *M. balthica* shells, this issue remains unsolved and open to further investigations.

Although the genetic basis of two aspects of colouration (hue and intensity) in *M. balthica* is thus shown, and that of a third (bicolouration) is suggested, the possible effects of environmental influences remain to be investigated. As noted by Cain (1988), prolonged burial in certain sediments can stain *M. balthica* rust-coloured or black, which changes the appearance of the innate colour. Creese & Underwood (1976) demonstrate that shell banding pattern in the marine snail *Austrocochlea constricta* differs between habitats and that transplanted shells adjust their banding pattern in the direction of patterns more typical of the new location. Underwood & Creese (1976) propose that this effect is due to a difference in food availability; animals living on substrate with higher chlorophyll concentrations develop darker banding patterns. The saturation level of colours of a wide range of molluscs has been shown to be related to concentration of pigment in the shell material (Underwood & Creese, 1976; Hedegaard *et al.*, 2006). Food abundance or quality could influence colour saturation if substrates for pigments are limited resources, as suggested by, e.g. Comfort (1951), Ino (1949), Leighton (1961) and Hedegaard *et al.* (2006). For *M. balthica*, the observed difference in shell colour intensity between adults and juveniles, dependent on colour class (Fig. 2), may be an indication of an environmental effect. Although rearing conditions were in principle the same for all our crosses, (micro-)environmental effects could play a role in *M. balthica* and help to explain the substantial amount of variation for colour intensity unaccounted for in the parent–offspring analysis (Tables 3 and 4; Fig. 3).

Simple genetic models such as the one proposed here are common for colour polymorphisms (Innes & Haley, 1977; Palmer, 1985; Ekendahl & Johannesson, 1997; Yusa, 2004). Not much is known about how the simple genetics translate into the alternative phenotypes. The biochemistry of molluscan shell colour is to a large extent still unknown territory. A number of colours are known to be produced by polyenes (Hedegaard *et al.*, 2006), which are poly-unsaturated carbohydrates, while porphyrins, which are organic compounds containing four pyrrole rings, also occur (Underwood & Creese, 1976; Jones & Silver, 1979). Qin *et al.* (2007) identified several genetic markers based on AFLP (Amplified Fragment Length Polymorphism) that were all in the same linkage group and associated with inheritance of the colour orange, which is dominant over white, in the bivalve *Argopecten irradians*. This is evidence for a single physical locus determining the orange colour, and it seems likely that the white genotype lacks deposition of pigment in the shell altogether and hence has no pigment. Further elucidation of the chain of events from the nature of this DNA polymorphism to the formation of orange colour in *A. irradians* shells will be very helpful to answer questions related to the role of limiting natural resources and colour production costs.

Looking at the occurrence of a polymorphism across populations and beyond species boundaries is insightful for judging the roles of mutation, genetic drift and selection (Harvey & Purvis, 1991). Here, colour polymorphism is studied in *M. balthica* from the northeastern Atlantic Ocean. The polymorphism is to an extent present within the species as a whole as well as in related taxa. The taxon *M. balthica* is regarded as a species complex (Väinölä & Varvio, 1989; Luttikhuizen, Drent & Baker, 2003b; Väinölä, 2003; Nikula, Strelkov & Väinölä, 2007), with subgroups that differ somewhat in colour frequencies. For example, *Macoma petalum* of the western Atlantic lacks yellow in most populations (Beukema & Meehan, 1985). *Macoma balthica* '*balthica*', in the Baltic Sea and White Sea, has relatively many red shells (Väinölä & Varvio, 1989; Strelkov *et al.*, 2001; Sokolowski *et al.*, 2002). Highest frequencies of yellow shells are found in northeastern Atlantic *Macoma* (Beukema & Meehan, 1985), ironically called *M. balthica* '*rubra*' (*ruber* is Latin for red). Geographical differentiation in colour frequencies within subgroups has also been reported: a cline of increasing yellow towards the south was described by Beukema & Meehan (1985) for *M. balthica* '*rubra*', and clines of the frequency of red at the entrances to Baltic Sea and White Sea in *M. balthica* '*balthica*' by Väinölä & Varvio, (1989) and Strelkov *et al.* (2001), although the latter might also be the result of hybridization with *M. balthica* '*rubra*'. Moving to higher taxonomic scales, within the family Tellinidae to which *M. balthica* belongs, several other species can be found with a similar colour polymorphism, e.g. *Tellina tenuis* and *Tellina pygmaea* (Cain, 1988); mostly white and red are present, sometimes orange and rarely yellow. In contrast, some species in the Tellinidae are monomorphic for white, e.g. *Macoma nasuta* (Cain, 1988; Oldroyd, 1924). However, colour polymorphism is found once more in related families belonging to the Tellinoidea. For example, some *Gari* species (Psammobiidae) and *Donax* species (Donacidae) have red, orange, yellow and even purple shells. These observations seem to suggest that this is a 'protected polymorphism', i.e. rare morphs have elevated fitness and are therefore protected from being lost through genetic drift, which is why the polymorphism may have been maintained over long evolutionary time scales, surviving population differentiation and speciation. The possible involvement of balancing selection forces (Cain, 1988; Hiddink, 2002) as well as an analysis of mutation/drift balance in the maintenance of *M. balthica* colour polymorphisms should be subjects for continued studies.

Several selection factors have been suggested to underlie molluscan shell colour polymorphisms. An example of an abiotic factor is temperature; dark-shelled *Mytilus* species can heat up in the sun 2–3°C more than lighter morphs (Mitton, 1977). Also salinity (e.g. in *Littorina saxatilis*: Sokolova & Berger, 2000) and protection against UV radiation (e.g. in *Theodoxus jordani*: Heller, 1979) have been demonstrated to be possible abiotic selection factors. Predator avoidance by crypsis, or the matching of the phenotype with substrate colour or pattern, is a biotic adaptive attribute that colours and patterns of shells can convey (e.g. Smith, 1975; Heller, 1979; Reid, 1987; Whiteley, Owen & Smith, 1997; Johannesson & Ekendahl, 2002; McKillup, McKillup & Pape, 2000). However, in order to explain specifically the maintenance of a polymorphism, as opposed to evolution towards monomorphy, frequency-dependent selection factors must be involved. Crypsis might result in polymorphism if the habitat is heterogeneous (Whiteley *et al.*, 1997; Rodrigues & Absalão, 2005), but true frequency dependent selection stems from apostasis; the preference of individual predators (or parasitoids) for a particular morph, e.g. through the formation of a search image (Smith, 1975; Reid, 1987; Cain, 1988; Whiteley *et al.*, 1997; Bond, 2007). The only life stage during which the polymorphism studied here is visible is when the juveniles are several millimetres long and undertake secondary migration by the use of a mucous thread, and it has been suggested that visual hunting by predatory fish might constitute a balancing selection force (Hiddink, 2002). An alternative to the idea that colour polymorphisms serve some adaptive purpose is neutrality (Comfort, 1951; Cox *et al.*, 1969; see for a non-molluscan example: Hoffman *et al.*, 2006). Cox *et al.* (1969) view colour polymorphism in the majority of bivalves that are burrowed in sediment as without function and the result of waste products of metabolism.

The demonstration of a genetic basis of *M. balthica* shell colour polymorphism that is invisible throughout most of the clams' lives will provide the basis for further analysis of the evolutionary forces that maintain the polymorphism. Furthermore, the proposed genetic model makes of the colour variation in this species a readily available genetic marker for addressing population genetic questions.

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