

GENETICS OF PIGMENTATION IN *PORCELLIO SCABER* LATREILLE, 1804
(ISOPODA, ONISCIDEA)

BY

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ABSTRACT

In the sowbug, *Porcellio scaber* Latreille, 1804 (Isopoda, Oniscidea), the genetics of carapace and eye colour were investigated. Carapace shield colours studied include white, dark grey/brown, light orange, and a variegated phenotype consisting of an orange background with darker brownish pigmented regions. Eye colours found include colourless, red, and black. Two loci, one with two alleles the other with three alleles, can explain the patterns of inheritance we found in the carapace shield and eyes. Alleles at a single locus with incomplete dominance appear to control carapace edge colour. Interactions between some alleles at different loci were discovered and correlations between eye and carapace colours are described. Variegation was limited to females and not expressed in males of similar genotype.

ZUSAMMENFASSUNG

An der Kellersassel, *Porcellio scaber* Latreille, 1804 (Isopoda, Oniscidea), wurde eine Studie zur Genetik der Pigmentierung von Carapax und Augen durchgeführt. Die untersuchten Phänotypen für den Schild des Carapax schließen einen weißen, hell orangefarbenen und dunkel graubraunen Pigmentierungsphänotyp ein, sowie einen gesprenkelten Phänotyp mit dunkelbraunen Pigmentflecken auf orangefarbenem Hintergrund. Die Augen der untersuchten Asseln waren entweder farblos, rot oder schwarz. Die aufgedeckten Vererbungsmuster können durch zwei Genloci mit zwei, beziehungsweise drei Allelen erklärt werden. Allele eines dritten Locus beeinflussen die Pigmentierung am Außenrand des Carapax und zeigen unvollständige Dominanz. Zwischen einigen Allelen der verschiedenen Genloci gibt es epistatische Wechselwirkungen. Beziehungen zwischen der Pigmentierung des Carapax und der der Augen werden beschrieben. Der gesprenkelte Phänotyp war auf weibliche Asseln beschränkt und wurde in männlichen Asseln vom gleichen Genotyp nicht exprimiert.

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INTRODUCTION

Polymorphisms within natural populations have provided basic data for understanding the scope of genetic variation that underlies evolutionary change. Indeed, the documentation of the vast amount of often hidden genetic variation is perhaps the most important legacy of 20th-century population genetics. How this reservoir of variation is maintained in populations is still unresolved (Franklin & Lewontin, 1970). Rare, but persistent polymorphisms are often reported for organisms that have not been analysed genetically and these polymorphisms may be profitably studied to determine factors contributing to their maintenance, whether it be selection under certain environmental conditions, sexual selection, or drift. *Porcellio scaber* Latreille, 1804 (Isopoda, Oniscidea), a terrestrial isopod probably introduced to North America from Europe (Jass & Klausmeier, 2000), has a variety of naturally occurring polymorphisms that could be useful in such explorations. However, prior to examining allele frequencies at the population level, it is necessary to distinguish alleles within the organisms studied and we present here our current understanding of the genetics of eye and carapace coloration in a population of *Porcellio scaber* collected from a compost heap in Toronto, Canada, and maintained for several years as a colony in large terraria on a diet of rabbit pellets and carrots, supplemented with chalk. Colour variants were culled from the main colonies and reared separately. These formed the starting material for our genetic analysis.

P. scaber is commonly a uniform grey or dark brownish colour (henceforth called grey) with black eyes. With continued inbreeding we have found a light coloured carapace edge polymorphism, carapace colours ranging from orange to white including mottled and speckled patterns, and eye pigmentation including black, orange, and light. We have not found sex-linked traits but do find that a mottled phenotype is only expressed in females although it appears to be transmitted by males (sex-limited trait). We have found evidence for several interacting loci, some with multiple alleles, affecting both carapace and eye colour.

METHODS

Rearing

Plastic containers of 500 or 250 ml were used to house isopod stocks and crosses. A laundered Kimtex™ towelling strip was placed in the bottom of each container and through a small hole (to absorb water from the bottom of a wallpaper tray fitted with two bars on which the containers sit). Dirt was packed on top of the Kimtex™ and bark or flexible plastic was provided to serve as hiding places.

Rabbit pellets (1-5) were fed weekly or bi-weekly in a small plastic Petri dish and a small piece of carrot and chalk were added once or twice a month. A 16-hour photoperiod, to enhance reproduction, was approximated by the addition of fluorescent lights and an incandescent lamp controlled by a light sensitive timer to go on when room lights were turned off.

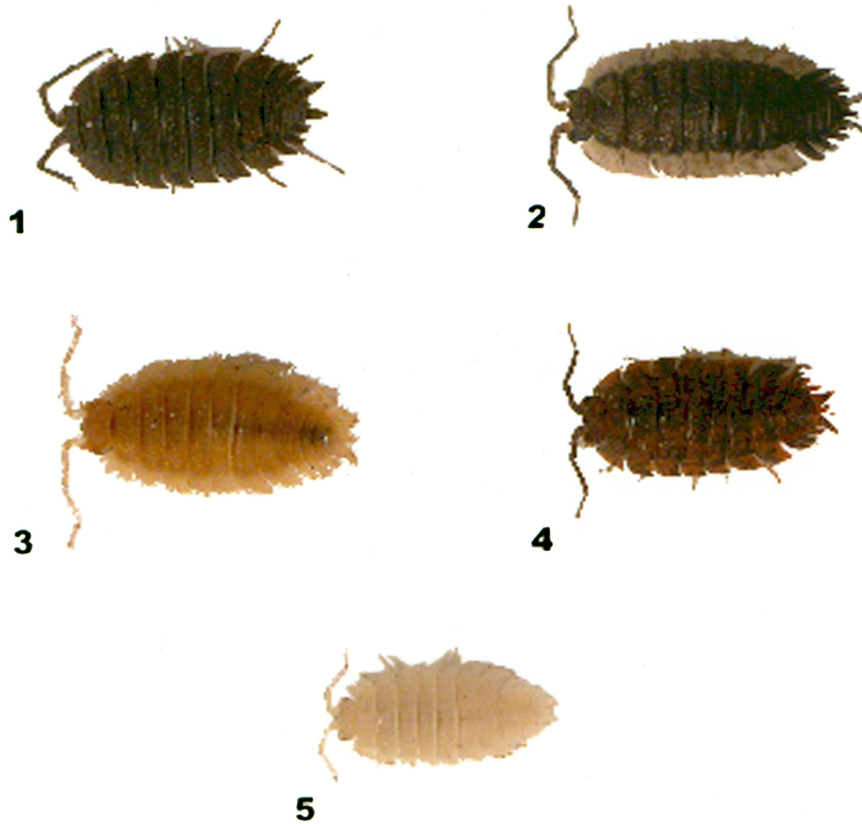
Crosses

Porcellio scaber is a communal breeder, so crosses involved up to 5 virgin females with one male in a container. It was important that females were virgins because terrestrial isopods are known to retain viable sperm for long periods (Sassaman, 1978). Animals were sexed and separated at about 8 mm long (2 to 3 months of age) before the maturity of the females, which occurred at around 4 to 5 months. If desired, pregnant females were isolated prior to birth of their progeny, thus effectively achieving single pair matings. Pregnancy lasts about 20 days at 25°C to 60 days at lower temperatures (Warburg, 1993); in our colony, temperatures probably did not go below 20°C. White mancas are born live and they can be colour and sex classified at a length of about 8 mm. In the case of mottled phenotypes, certainty of classification might be delayed until 5 months of age. Sexing and colour determination required magnification using a stereo microscope, and sometimes anaesthesia with CO₂ gas. The descriptions of isopod anatomy by Oliver & Meecham (1993) were used for sex determination. Where feasible, reciprocal crosses were made.

Limitations on interpretations arise from the fact that our lines are not completely pure breeding and broods for each female at the time of phenotypic analysis are typically less than 40. Because pooling results may obscure interpretation if the females were not all of the same genotype, pooled results are identified in the text. Otherwise, data are from separated pairs.

Phenotypes

Figs. 1-5 of pl. 1 present the phenotypes we studied in our colony. The predominant phenotype in nature is the grey carapace phenotype with black eyes shown in fig. 1, but there is also a white-edged variant as shown in fig. 2. Only black eyes have been seen on grey isopods. A phenotype with light orange carapace, a white carapace edge, and red eyes is seen in fig. 3; a light orange carapace always appeared with red eyes and light carapace edge. Virtually white carapace individuals (called albino, in future) with either light (un-pigmented to a light orange cast) or black eyes have also been studied; edges were the same colour as the rest of the carapace (fig. 4). Albino carapaces are sometimes decorated with a few darker patches. Variegated phenotypes of two types have been observed; the



Pl. 1 figs. 1-5, colour morphs of *Porcellio scaber* Latreille, 1804. 1, common grey/brown carapace coloration; 2, grey/brown carapace shield with white edge; 3, orange carapace always seen with a white edge; 4, mottled carapace has a deeper orange background than seen in fig. 3 and has superimposed irregularly placed brown regions; 5, albino carapace which sometimes display regions of small black spots.

one we refer to as mottled (fig. 5) has dark pigment scattered over an orange base, which is a deeper orange than in the orange morph. This carapace patterning is associated both with and without a white edge, but it is always found with black eyes. The other variegated phenotype is speckled, having a finer pattern of dark areas over an orange background. We have not studied this pattern genetically and will not discuss it further.

Statistics

Chi-square tests were performed to test genetic hypotheses against the data. Hypotheses were accepted if there were no significant differences between observed and expected values (5% level of significance). Where two hypotheses showed non-significant differences, the one with the smaller chi-square value is given.

RESULTS

We gathered genetic evidence for: grey, albino, and orange solid carapace shield colour; mottled carapace shield; light and dark carapace edge; and black, red, and light eye pigment. There were instances of occasional darker patches on light-coloured carapaces but these were not studied in detail from a genetic perspective. The data below were collected from quasi-inbred lines. Some of the phenotypes we eventually uncovered emerged in lines thought to be largely pure breeding, thus our results are a start to understanding the inheritance of natural genetic variation influencing coloration of an isopod.

Edge pigment

Although a carapace edge lighter in colour than the rest of the carapace was one of the first polymorphisms studied, it proved to be somewhat difficult because intermediates between light and dark were found and classification could be inconsistent depending on the age of the isopod observed or the sensitivity of the observer. In numerous crosses, white edge was at least incompletely dominant to dark edge. An F1 \times F1 cross from a white edge \times dark edge produced 88 not white-edged to 41 white-edged progeny, which is consistent with a 3:1 as well as a 2:1 hypothesis when using chi-square distribution suggesting a single locus, two allele hypothesis. White edge bred true and dark edge was never found with orange or identified in albino carapaces, although it was with mottled and speckled phenotypes as well as grey carapaces. Where edge colour segregated at all, it appeared to assort independently of carapace colour. Because of ambiguities in classification, the unexplored possibility of confounding lethality as well as apparent interactions with other loci (those responsible for light orange and albino phenotypes) in the carapace colour genetic pathway, we will not present data on edge colour in the crosses to be described.

Eye colour and carapace colour

Below are data that support the conclusions that eye colour is controlled by two loci called I and C, where black eyes are produced when at least one dominant allele b^+ is present at the I locus. When both alleles at the I locus are recessive (b^-/b^-), red eyes and orange carapace will occur if the C locus contains at least one a^+ allele; if the C locus is homozygous for the recessive a^- , as well as for b^- , light eyes will be seen. Therefore, alleles at the I locus interact with alleles at the C locus affecting both carapace and eye colour. Grey, black-eyed sow bugs contain an a^+ at the C locus and a b^+ at the I locus. Unless noted, females and males did not differ qualitatively or quantitatively. These relationships are summarized in

TABLE I
Genotype-phenotype relationships in carapace coloration of the isopod, *Porcellio scaber* Latreille

Phenotype	Required alleles or allele combinations	Forbidden alleles or allele combinations	Possible genotypes	
			C locus sex (females = ♀♀, males = ♂♂)	I locus
Grey carapace, Black eyes	$a^+/- b^+/-$ (in ♀♀, in ♂♂, a^v can substitute for a^+)	a^v (in ♀♀ only)	a^+/a^- or a^+/a^+ ; ♀♀ and ♂♂ a^v can substitute for a^+ in ♂♂	b^+/b^+ or b^+/b^-
Orange carapace, Red eyes	$a^+; b^-/b^-$	b^+	$a^+/(a^-$ or a^+ or $a^v)$; ♀♀ and ♂♂	b^-/b^-
Albino carapace, Black eyes	b^+	$a^+; b^-/b^-$	a^- or a^v/a^- or a^v/a^v ; ♀♀ and ♂♂	b^+/b^+ or b^+/b^-
Albino carapace, Light eyes	b^-/b^-	a^+ and b^+	a^-/a^- or a^v/a^- or a^v/a^v ; ♀♀ and ♂♂	b^-/b^-
Mottled carapace, Black eyes	$a^v; b^+$	b^-/b^-	a^v/a^- or a^+ or a^v ; ♀♀	b^+/b^+ or b^+/b^-

table I. In the data provided, putative genotypes are given in parentheses, a alleles are said to be at the C locus and b alleles at the I locus and a “-” means that there are no restrictions on which of the known alleles at the locus, occurs.

Cross A:

Grey carapace and Black eyes ♀ × ♂
 $(a^+/a^+; b^+/b^+)$
 ↓
 129 Grey carapace and Black eyes
 $(a^+/a^+; b^+/b^+)$

These results indicate that this grey carapace and black eyes stock is pure breeding.

Cross B:

3 Grey carapace and Black eyes ♀♀ × 1 Orange carapace and Red eyes ♂
 $(a^+/a^+; b^+/b^+) × (a^+/a^+; b^-/b^-)$
 ↓
 all Grey carapace and Black eyes
 $(a^+/a^+; b^+/b^-)$

Cross C:

$$\begin{array}{c}
 \text{F1 } \text{♀♀} \times \text{F1 } \text{♂♂} \text{ Grey carapace and Black eyes} \\
 (a^+/a^+; b^+/b^-) \\
 \downarrow \\
 69 \text{ Grey carapace and Black eyes } (a^+/a^+; b^+/b^-) \\
 26 \text{ Orange carapace and Red eyes } (a^+/a^+; b^-/b^-)
 \end{array}$$

Cross B suggests that grey carapace and dark eyes are dominant to orange carapace and red eyes and the three to one ratio in Cross C (chi-square value 0.28) and suggests that red eyes and orange carapace differ from black eyes and grey carapace by homozygosity of a recessive allele at one locus, the I locus.

Cross D:

$$\begin{array}{c}
 \text{F1 Grey carapace and Black eyes } \text{♀} \times \text{Orange carapace and Red eyes } \text{♂} \\
 (a^+/a^+; b^+/b^-) \times (a^+/a^+; b^-/b^-) \\
 \downarrow \\
 89 \text{ Grey carapace and Black eyes } (a^+/a^+; b^+/b^-) \\
 76 \text{ Orange carapace and Red eyes } (a^+/a^+; b^-/b^-)
 \end{array}$$

If the progeny of Cross B are indeed heterozygous at the I locus, Cross D should produce a 1:1 ratio of phenotypes and this hypothesis was accepted. These results lead to the hypothesis that one locus with two alleles can account for the transmission of eye and carapace colour. In similar crosses with different individuals, a small proportion of progeny (less than 4%) were speckled with black eyes. This suggests that other loci/alleles may interact in this system to produce modified phenotypes.

The next crosses tested the genetic relationship between sowbugs with albino carapaces and those with grey or orange carapaces.

Cross E:

$$\begin{array}{c}
 5 \text{ Grey carapace and Black eyes } \text{♀} \times \text{Albino and Black eyes } \text{♂} \\
 (a^+/a^+; b^+/b^?) \times (a^-/a^-; b^+/b^+) \\
 \downarrow \\
 \text{all Grey carapace and Black eyes } 16 \text{ } \text{♂♂} \text{ and } 10 \text{ } \text{♀♀} \\
 (a^+/a^-; b^+/b^?)
 \end{array}$$

Cross E shows that albino is recessive to grey carapace, the finding of light-eyed progeny in Cross F suggests that one of the parents from Cross E was heterozygous at the b locus (indicated by a question mark).

Cross F:

$$\begin{array}{c}
 \text{F1 Grey carapace and Black eyes } \text{♀} \times \text{♂ (from Cross E)} \\
 \downarrow
 \end{array}$$

69 Grey carapace and Black eyes ($a^+/-; b^+/b^+$)

37 Albino carapace and Black eyes ($a^-/a^-; b^+/b^+$)

8 Albino carapace and Light eyes ($a^-/a^-; b^-/b^-$)

The 2 : 1 ratio of grey to albino carapace in Cross F suggests that the difference between these carapace colours depends on homozygous recessive alleles for the albino carapace at the C locus but a 3 : 1 ratio would be easier to explain. The appearance of light eyes in Cross F led us to test that the F1 progeny of Cross E were indeed heterozygous at a single locus by crosses to a homozygous recessive albino individual.

Cross G:

F1 Grey carapace and Black eyes ♀ × Albino and Black eyes ♂
 (putative testcross for carapace colour)
 $(a^+/a^-; b^+/b^+) \times (a^-/a^-; b^+/b^+)$
 ↓
 14 Grey carapace and Black eyes ($a^+/-; b^+/-$)
 11 Albino carapace and Black eyes ($a^-/a^-; b^+/-$)
 1 Mottled carapace and Black eyes (?)♀

These limited data produced the expected 1:1 ratio for carapace colour, indicating a single locus with two alleles determining whether the carapace will be albino or grey. The unexpected appearance of the mottled phenotype was evidence for additional genetic variance for carapace coloration and the genetics of this phenotype are explored later. The occasional appearance of new phenotypes in otherwise “well behaved” stocks suggests that some phenotypes appear only under some genetic backgrounds, perhaps where suppressors are removed.

Cross H:

Orange carapace and Red eyes ♀ × Albino and Black eyes ♂
 $(a^+/a^-; b^-/b^-) \times (a^-/a^-; b^+/b^-)$
 ↓
 9 Grey carapace and Black eyes ($a^+/-; b^+/-$)
 12 Orange and Red eyes ($a^+/-; b^-/b^-$)
 11 Albino carapace and Black eyes ($a^-/a^-; b^+/b^-$)
 10 Albino carapace and Light eyes ($a^-/a^-; b^-/b^-$)

The limited 1:1:1:1 ratios from this cross suggest the existence of two independently assorting loci, each with 2 alleles, with interactions between loci such that orange carapace requires homozygous b^- in the presence of a^+ . Putative genotypes are provided parenthetically for each phenotype. The albino carapace and light eyes phenotype shows that eye colour can be uncoupled from carapace colour in this case and therefore the change in eye colour is likely due to alleles at

TABLE II

Cross I: mottled carapace is found only in females with an a^v allele and requires a b^+ allele

F1 phenotypes (putative genotypes)	Orange carapace, Red eyes ($a^+/-; b^-/b^-$)	Grey carapace, Black eyes ($a^v/-; b^+/-$)	Mottled carapace, Black eyes ($a^v/-; b^+/-$)	Albino carapace, Light eyes (Not $a^+; b^-/b^-$)	Total
Males	9	8	0	7	24
Females	6	0	8	3	17

the I locus. If our genotype assignments are correct, the albino carapace and light eyes phenotype is a tester strain (homozygous recessive) for the C and I loci and could be used to examine allele frequencies in natural populations.

Note that a dash ($/-$) in a genotype means that in the progeny of this cross, the same phenotype will appear with any of the possible alleles at that locus.

Genetics of mottled carapace

We have observed over 85 mottled isopods in various crosses and all have been female with one exception that did not live long enough to be re-scored at a mature stage, so we believe this trait to be sex limited. Crosses with sons of mottled isopods to non-mottled females have produced mottled daughters, implying that the trait may be inherited through the male line.

The data to be presented fit best with the following hypotheses in which the C locus contains at least three alleles and the I locus, two alleles. The C locus allele a^v (for variegation) promotes mottling in one dose and is dominant to a^+ and a^- in the presence of a b^+ allele at the I locus (see table I), but only in females. Mature males do not show the mottling effect. The data shown are from broods from single females because different mottled females can have different genotypes and males apparently can carry but not express mottling. Although the brood sizes are small, the observed phenotypes and their ratios are supportive of the relationships in table I.

Cross I:

Mottled carapace, Black eyes ♀ × Orange carapace, Red eyes ♂

($a^v/a^v; b^+/b^-$) × ($a^+/a^-; b^-/b^-$)

(see table II)

These progeny demonstrate the finding encountered in several crosses, that mature males are not mottled and suggest that females may display mottled

TABLE III

Cross J: further evidence for sex limited expression of the mottled phenotype

F1 phenotypes (putative genotypes)	Mottled carapace, Black eyes $a^v/a^v; b^+/b^-$ or $a^v/-; b^+/b^-$	Grey carapace, Black eyes $a^+/a^-; b^+/b^-$ ♀♀ or ♂♂ $a^v/-; b^+/b^-$ ♂♂ only	Total
Males	0	44	44
Females	36	12	48

TABLE IV

Cross K: evidence for male transmission of mottling allele it does not express

F1 phenotypes (putative genotypes)	Mottled carapace, Black eyes $(a^v/a^+; b^+/b^-)$	Grey carapace, Black eyes $(a^+/a^-; b^+/b^-)$ ♀♀ or ♂♂ $(a^v/a^+; b^+/b^-)$ ♂♂ only	Total
Males	0	23	23
Females	15	13	28

phenotypes where males of the same genotype produce grey carapaces. In this cross, grey carapace, black eyed females; mottled carapace, red eyed females as well as albino black eyed phenotypes of either sex were neither predicted nor seen. The data are not numerous enough for statistical tests of the predicted ratios; 2 mottled black eyes : 1 orange red eyes : 1 albino light eyes phenotypes among females. We hypothesized that the mottled carapace required another allele at the C locus, a^v , which is dominant to a^+ .

The data in Cross J are the pooled results from matings of one male to two mottled females presumed identical at the C and I loci because their offspring are qualitatively similar. In these crosses, a 3 : 1 ratio of mottled to grey carapace was predicted and found in female data. Also as predicted, males were exclusively grey. The albino male in this cross is thought to carry the a^v allele because of the results of Cross K in which it produced mottled daughters in a cross lacking a mottled mother.

Cross J:

Mottled carapace and Black eyes ♀ × Albino carapace and Light eyes ♂

 $(a^v/a^+; b^+/b^+) \times (a^v/a^-; b^-/b^-)$

(see table III)

TABLE V

Cross L: test cross results are consistent with predictions based on putative genotypes

F1 phenotype (putative genotypes)	Mottled carapace, Black eyes ($a^v/a^-; b^+/b^-$)	Grey carapace, Black eyes ($a^v/a^-; b^+/b^-$)	Albino carapace; Black eyes ($a^-/a^-; b^+/b^-$)	Albino carapace; Light eyes ($a^-/a^-; b^-/b^-$) or ($a^v/a^-; b^-/b^-$)	Total
Males	0	3	2	6	11
Females	4	0	3	7	14

Cross K:

Grey carapace and Black eyes ♀ × Albino carapace and Light eyes ♂
 $(a^+/a^+; b^+/b^+) \times (a^v/a^-; b^-/b^-)$
 (see table IV)

The hypothesis of male transmission was tested in cross K using the male from Cross J (derived from a cross of a mottled black eyed female and albino light eyed male, a grey F1 male was then crossed to an albino light eyed female producing the male used here). The existence of mottled females in progeny of this test cross is consistent with transmission of the variegating allele through males.

Cross L:

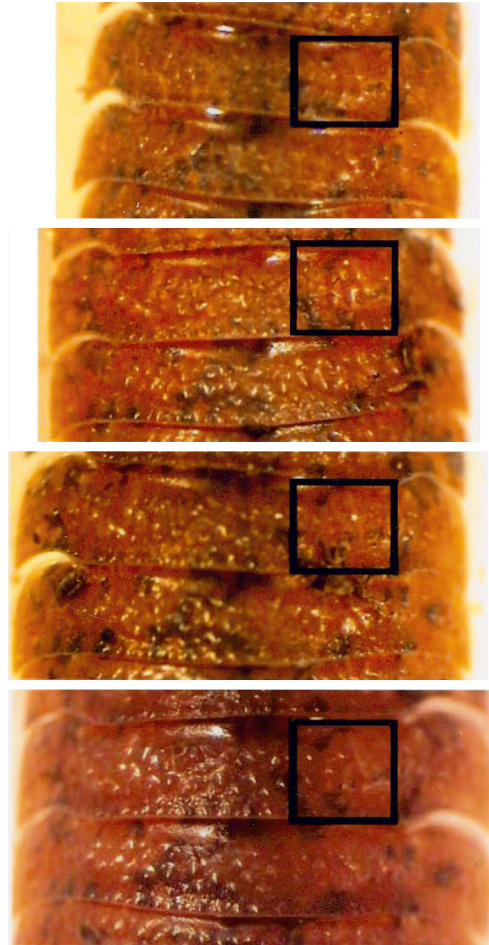
Albino carapace and Light eyes ♀ × Grey carapace and Black eyes ♂
 $(a^-/a^-; b^-/b^-) \times (a^v/a^-; b^+/b^-)$
 (see table V)

Despite the small sample size, two predictions from the putative genotype assignments are seen in the data. There are no females with grey carapace and black eyes (no a^+ alleles) and there are about twice as many albinos with light eyes as there are with black eyes (because two genotypes give this phenotype).

Patterns of mottling were found to change in females with successive moults. This is illustrated in pl. 2, in which the carapace of the same mottled female was photographed after different moults. The implication is that this represents an instability in gene expression not only spatially but also temporally.

DISCUSSION

Colour variations apparently similar to those discussed here have been described by field naturalists in European as well as North American *Porcellio scaber* populations, so it is possible that they have been maintained by selective advantage.



Pl. 2. Photographs of the same mottled somites of *Porcellio scaber* Latreille, 1804, over a period of several moults. The box encloses a sample area in which the location of brown pigmentation is shown to change subtly with age.

Isopods are generally hidden from view during the day (Warburg, 1993) particularly under desiccating conditions but they move around opportunistically. No correlations have been found between pigment patterns and factors such as moisture preferences, mating preferences, waste deposition mechanisms, or thermal regulation, in another species, *Venezillo evergladensis* Schultz, 1963 (cf. Johnson, 1984). Pigment polymorphisms have been reported in several species of terrestrial isopods (Howard, 1940; Johnson, 1984; Hasegawa et al., 1997; and references therein). Johnson's work on *Venezillo evergladensis* (summarized in Johnson, 1984) showed results similar to our own, for example, there were unlinked autosomal loci with multiple alleles at one locus and epistatic interactions between certain alleles of

particular loci. He reported red morphs as well as brownish and variegated morphs (Johnson, 1983) and found correlations between some carapace colours and eye colours.

Sex limited, variegated colour phenotypes in females were reported in *Armadillidium vulgare* (Latreille, 1804) by Howard (1940), who reported that females were the heterogametic sex. We do not know if *P. scaber* has sex chromosomes and if so, which sex is heterogametic. Legrand et al. (1987) described two subspecies of *P. dilatatus* Brandt, 1833, in which the male was the heterogametic sex in one subspecies while it was the female who was heterogametic in the other. Regardless of potential chromosomal sex determination, hormonal milieu has been found to influence (although not determine) sex in isopod transplant experiments (Suzuki, 1999); therefore, we hypothesize that sex limited variegation may be female limited owing to favourable hormonal conditions. The appearance of red as well as dark (brown, grey) and variegated colour morphs in terrestrial isopods belonging to different families, as well as some of the genetic similarities in their control suggest that there may be ancient themes involving similar biochemical synthetic pathways. The spatial regulation of their deposition may be different in different species. For example, we did not notice, as Johnson (1984) did, colour deposition patterns in antennae different from carapace colour. Too little information on the biochemical pathways involved in isopod coloration is available to suggest detailed hypotheses concerning the genetic control of colour patterning. Crustacea are known to contain a large complement of pigment molecules (Needham, 1974) and studies in isopods have illustrated that they have ommochromes that can produce a variety of red and dark pigments (Hasegawa et al., 1997). A testable hypothesis is that ommochromes and/or melanistic pigments are produced with and mask orange pigments. The selective removal of darker pigments may reveal orange colours of various intensity, while removal of all pigments would lead to the white (albino-like) phenotype. Quantifying different classes of pigments during development and in the adult carapace could help to clarify if colour variation in isopods results from quantitative regulation of the synthesis of different pigments only, or if differential uptake (or a combination) is involved.

There are distinct differences between aspects of colour patterning of insects such as *Drosophila*, and these terrestrial isopods. In flies, colours may deepen with age but patterns do not change because there is no moulting after metamorphosis to the adult stage. This is not so in our isopods, in which the variegated phenotype changed subtly with moulting over the course of months. Other workers have noticed changes in eye coloration with age (Johnson, 1983) but we are probably the first to document the change in variegation pattern with successive moults. Variegation, particularly position effect variegation, has been well studied in the insect, *Drosophila melanogaster* Meigen, 1830 (cf. Tartof, 1989) but as mentioned,

adults of such holometabolous insects do not moult and the pattern of variegation appears fixed, shortly after adults emerge from the pupal case. Changes with age in genetically controlled variegation patterns have been documented in mice where hair replacement cycles can be followed (Cattanach, 1974). In mice, variegation is associated with the position on the chromosome of pigment associated genes. It is thought that gene activation may be controlled by local chromosome structure (heterochromatin) and that some loci may be in a region of changing structure in different cell cycles. Sometimes, variegation is associated with the presence of repeated gene arrays, which are thought to create local heterochromatic regions that in turn modulate local gene expression (Dorer & Henikoff, 1997). It would be interesting to determine whether the variegation in isopods we have observed is a naturally occurring example of chromosomally based variegation and how it is maintained in the population.

The albino-like phenotype is of considerable interest, since mancae are initially albino before acquiring pigmentation, whereas a documented isopod species with a subterranean existence, *Platyarthrus hoffmannseggii* Brandt, 1833 living in ant nests, maintain albino cuticles into adulthood. Because some of our white sow bugs have black eyes and dark spots on their carapace, we suspect that the albinism is not due to a genetic deficiency in pigment production but rather to a difficulty in transporting pigment precursors into some tissues. A similar situation exists with the gene for white eyes in *Drosophila*. Here, a defective ABC transporter has been identified (Sullivan & Sullivan, 1975).

As indicated earlier, we are not aware of compelling evidence for the adaptive significance of colour variation in isopods in general and in *Porcellio scaber*, in particular. Johnson (1984) tested colour morphs of *Venezillo evergladensis* for variation in desiccation tolerance and found no differences. In addition, with the exception of one genotype, all appeared equally fecund and robust. He did, however, find geographical variation in the distribution of colour morphs for which no obvious selective basis was suggested. The colour variations in *P. scaber* may currently be adaptively neutral but very ancient, and the alleles associated with them may have arrived in North America when *P. scaber* was introduced there and been maintained, perhaps by chance. The genotypic analysis described here can provide a basis for exploring a number of hypotheses relating to the maintenance of genetic variation in natural populations. If the genotypes are adaptively neutral over a range of environments, there should be no difference among them in their thermal and desiccation tolerance or their sensitivity to light. Furthermore, no differences between them with respect to mating preference should be found.

Strains that are homozygous recessive for gene loci of interest will reveal the presence of both dominant and recessive alleles when bred to an individual with

unknown genetics. Recessive “tester” strains such as the pure breeding albino light eyed sowbugs could be used to estimate allele frequencies at C and I loci in natural populations to further explore geographic or climatic considerations in the maintenance of these colour polymorphisms. Using table I, the genotype of offspring can be inferred from their phenotypes and knowing the genotype of the recessive parent will allow determination of the genotype of the other parent.

Future work on *P. scaber* pigmentation will include exploring the relationship between red eyed phenotypes of orange and the light eyed phenotypes of albino animals to determine if there are qualitative or quantitative differences of accumulation of pigment with age. Further work is also needed to clarify the basis for the patches of darker colours occasionally seen on otherwise albino carapaces to determine if these are somatic in origin or are influenced by germline genes.

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