COLOUR POLYMORPHISM IN THE LAND ISOPOD *ARMADILLIDIUM NASATUM**

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Received 7.vi.68

1. INTRODUCTION

Armadillidium nasatum Budde-Lund is one of the commonest oniscoid isopods in the south-eastern United States (Schultz, 1961). In Albemarle County, Virginia, and probably throughout its range, the species is polymorphic for body colour. The Oniscoidea as a whole are quite variable in colour and pattern (Vandel, 1960, 1962), and several workers have studied the genetics of individual loci (for references see Vandel, 1945, 1962). Only two have dealt with the frequency of genes in populations. Howard (1940, 1953, 1962) has investigated the genetics of *Armadillidium vulgare* with some attention to populations. De Lattin (1939, 1951, 1954) has studied the genetics of albinism and marbling in many species and has determined gene frequencies in some of them. However, neither study has seriously attempted to discover the causes for the polymorphisms. An extensive literature is available on the taxonomy (Vandel, 1960, 1962), physiology (Edney, 1954), and ecology (Hatchett, 1947; Brereton, 1957; Paris, 1962, 1963; Warburg, 1965) of land isopods. These works provide all the necessary background for investigations of population genetics in this large and widespread group of animals. Therefore, it seems important to apply to *A. nasatum* the techniques of ecological genetics so successfully used for other animals (Fisher and Ford, 1947; Cain and Sheppard, 1954; Dowdeswell, 1961).

2. METHODS

(a) Collections

All of the isopods used in this study were collected from natural populations in Albemarle County, Virginia, from September 1964 to May 1968. The study area is a rural one of rolling hills with pasture and mixed hardwood forests. The climate is temperate with about three inches of rain each month. In Virginia, *A. nasatum* inhabits the partially shaded areas around buildings, usually with lawn grasses and shade trees. It is rare in forests and open grasslands.

Most of the collections were taken from aggregations of isopods beneath a piece of cover such as a log or stone. After an object was raised, the animals clinging to it were brushed into a pan and the animals in the soil were scooped up with a spoon. Since the procedure did not rely on a visual search for individuals, a colour bias in the sampling was unlikely. The animals were taken to the laboratory and kept at 7° C. until scored. Holding at this low temperature reduced mortality considerably.

* Supported in part by an NDEA Title IV fellowship and in part by a Virginia Mason Davidge fellowship; part of a dissertation presented to the University of Virginia.

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(b) *Scoring*

Each animal was examined under a dissecting microscope and scored for colour, sex and body length. The length from the tip of the epistome to the tip of the telson is a reliable measure of the isopod's age. This distance was measured to the nearest millimetre by positioning the animal over a grid. No reference standard for colour was needed because the red class was quite distinct, but reliability was checked by scoring some collections twice. The two scores generally agreed ± 1 per cent. Sex was determined by the shape of the pleopods, which are rectangular in females and pointed in males. Only individuals 5 mm. or longer were sexed. Below that size, the male's pleopods are not completely differentiated.

(c) *Culture methods*

Adults were kept in 6-in. Petri dishes with discs of moist Whatman No. 1 filter paper on the bottom and a folded piece of the same paper in the centre. Breeding stock and young were kept in 4-oz. glass jars with dry paper on the bottom. The tops were fitted with air holes and shell vials that were filled with water and stoppered with cotton. This arrangement kept the humidity high while avoiding free water. All cultures were fed blackboard chalk and slices of potato and carrot. Changes in diet did not affect the body colours.

3. PHENOTYPES

Observation of individuals from natural populations and of young reared in the laboratory shows that body colour in *A. nasatum* varies both quantitatively and qualitatively. The quantitative variations are a normal result of the life-cycle; but the qualitative variations constitute, as this study will show, a genetic polymorphism. Three colour forms occur in natural populations. One is reddish-brown, here called red, very similar to human "red" hair. The colour referred to as "dark" can be either black or chocolate brown. In all forms the eyes and a region around the mouth are black. Whether black and brown are genetically different cannot be determined at present because the two cannot be distinguished reliably. For the purposes of this study, red is contrasted to dark. Intermediate phenotypes do occur, but not so frequently as to make scoring difficult.

All young are born colourless with red eyes and begin to show the body colour at about 2 months of age. Adults are darker than young, and males are more heavily pigmented than females. In adult males, the vas deferens has the same pigment as the epidermis and appears as a spot of colour through the ventral cuticle of the seventh thoracic segment. Almost all dark young, and some adults, have an area of pale red on the rim of the first thoracic segment. Occasionally, all the segments have red on the rims, giving a lightly pigmented animal an intermediate appearance. Three true mosaics were collected, two females and a male, with the normal dark pigment on half the seventh thoracic segment replaced by red. One of the females produced a segregating brood (Spot in table 1), indicating that she was heterozygous. Whatever the basis of the mosaicism, the animals demonstrate that both red and dark pigments can exist in the same body, unlike some mutant eye colours in insects.

Previous studies have shown that the epidermal pigments of *Asellus*

(Needham and Brunet, 1957) and *Idothea* (Lee, 1966) are ommochromes. However, both species are aquatic, and the pigments do not vary. Experiments with *A. nasatum* show that the pigments of this species are also ommochromes. Extractions were made either from single isopods or from batches carefully matched for colour. The animals were always decapitated to eliminate the eye pigments. The extraction procedure of Needham and Brunet was followed. First the bodies were ground in acetone and the colourless supernatant discarded. Next the tissue was ground in acid methanol (1 per cent. by volume HCl). The debris was centrifuged down and the supernatant used with no further purification. Extracts from black

TABLE 1

Matings of wild-caught virgins

Female	Male	Young born	Young scored Dark : Red = Total	Expected ratio	Significant at 5% level
RW1	RM1	19	0 : 15 = 15	0 : 1	*
RW2	RM1	10	0 : 8 = 8	0 : 1	—
RW9	RM1	8	0 : 3 = 3	0 : 1	—
RW3	RM2	19	0 : 15 = 15	0 : 1	*
RW4	RM3	12	0 : 11 = 11	0 : 1	*
RW5	RM3	8	0 : 4 = 4	0 : 1	—
RW6	RS1	16	0 : 9 = 9	0 : 1	—
RW8	RS2	11	0 : 5 = 5	0 : 1	—
IW1	DS1	14	4 : 4 = 8	—	—
IW2	DS1	12	7 : 0 = 7	—	—
IW3	DS1	11	8 : 3 = 11	—	—
IW4	DS2	13	4 : 2 = 6	—	—
IW5	DS2	20	7 : 3 = 10	—	—
DW1	DS3	17	10 : 3 = 13	3 : 1	*
DW2	DS3	10	1 : 1 = 2	3 : 1	—
DW3	DS3	19	7 : 4 = 11	3 : 1	—
Spot	?	21	2 : 4 = 6	—	—

The letters R, I, and D refer to the colour of the animals, red, intermediate, or dark. W signifies wild-caught virgin. M and S refer to the population from which the animals were taken, Murray or Searls. The female "Spot" was a non-virgin mosaic.

animals were wine red, from red animals were yellow, and from brown animals were orange. Scanning with a double-beam spectrophotometer produced the absorption spectra shown in fig. 1. A mixture of extracts from red and black was the same colour as an extract from brown and gave an identical spectrum.

The pigments had the chemical properties of ommochromes (Needham and Brunet, 1957; Lee, 1966). They were extracted by acid methanol, formic acid, aqueous HCl, and aqueous urea (10 per cent. w/v) but not by water, methanol or acetone. They showed a reversible colour change upon oxidation. After addition of sodium nitrite, all extracts turned brilliant yellow, a different shade from fresh extracts of red animals. When sodium borohydride was added, the oxidised solutions became pale pink, but the original colours were not restored.

To determine whether the solutions contained more than one pigment, ascending chromatograms were made on Whatman No. 1 paper using concentrated formic acid (fig. 2). When 0.05 ml. of methanol extract was applied to the paper, the colour was clearly visible. The spot with $R_f = 0.8$ was yellow and fluoresced in ultraviolet light. The spot with $R_f = 0.6$ was

pink and not fluorescent. After the yellow spots from red and black animals were eluted with acid methanol, they gave spectra identical to each other and to fresh extracts from red animals. Extracts from black individuals of *A. vulgare* gave chromatograms indistinguishable from those of black *A. nasatum*, suggesting that the pigment systems are the same. No red *A. vulgare* were available.

FIG. 1.—Absorption spectra of acid methanol extracts from isopods with (1) black, (2) red, and (3) brown phenotypes together with the spectrum (4) of a mixture of red and black; all normalised at 360 mμ.

Clearly, red is the absence of a dark pigment, and the observation that dark animals occasionally have some red on their bodies is consistent with the hypothesis that they normally have two pigments with the darker masking the lighter. Black and brown appear to differ only quantitatively. Needham and Brunet (1957) mention a wine-coloured pigment that they regard as an artifact or degradation product. However, the absence of the wine pigment in extracts from red animals strongly suggests that it is a natural pigment.

4. GENOTYPES

Two breeding programmes clarified the genetics of red. Each was dictated by the fact that females store sperm. In one, gravid females were

collected from natural populations and the segregation ratios of their broods determined. Since these young have not mated successfully, another programme using wild-caught virgins was begun. In both cases, separate stocks were taken from the Searls population (site A in fig. 3, frequency of red = 0.20) and the Murray population (site A in fig. 3, frequency of red = 0.36). The young were scored for colour at the age of 3 months and for sex at 7 months. Tables 1 and 2 give the results. Clearly, red behaves as a recessive trait.

FIG. 2.—Tracing of an ascending chromatogram of the extracted pigments; the difference in R_F values between brown and black is within the range of variability for a single extract.

The success of the matings shown in table 1 depended on the virginity of the females. To insure this, the stocks were collected in May before the breeding season had begun. Only females between 7 and 8 mm. long were used. A study of population dynamics (Adamkewicz, in preparation) shows that larger females may be in their second breeding season while smaller ones are not sexually mature. Moreover, some of the females were isolated from males, and these did not produce young. This control excludes the possibility of parthenogenesis or prior mating. The results also confirm that the females must have been virgin when collected. Whatever the dominance, the probability that eight red females that had mated in nature would all produce non-segregating broods is vanishingly small.

The matings of intermediate females with dark males (table 1) give some information on the status of brown and black. The males DS1, DS2, and DS3 were all definitely black, yet they must have been heterozygous for red. The females were all light brown, yet the results suggest that female IW2 was homozygous dark. Although brown may be a separate genotype, it clearly is not the heterozygote of red and black.

FIG. 3.—Map of the study area showing the percentage of red for 1964-66 at those sites where at least 100 isopods were collected.

Of the broods in table 2, only three are incompatible with the hypothesis that red is recessive. Two of these, red females whose broods segregated 3 dark : 1 red, were probably dark females misclassified as red. The third was a red female whose brood segregated 21 red : 6 dark. This animal came from the Murray population, and red Murray females as a whole had

TABLE 2
Assignment of broods from wild-caught gravid females

	Segregation ratios—Dark : Red					
	0 : 1	1 : 3	1 : 1	3 : 1	1 : 0	
Female parent						
Murray Red	0 : 24 0 : 7	6 : 21*	11 : 15 9 : 19 2 : 4 2 : 6 10 : 11 6 : 14 2 : 2	11 : 2*		
	0 : 7		2 : 2 11 : 13 7 : 12 1 : 4 7 : 10 10 : 16			
	Total 0 : 38		Total 81 : 125*			
Searls Red	0 : 16 0 : 7		8 : 10 6 : 9 4 : 3 9 : 7 1 : 2 9 : 10 3 : 3 2 : 7	10 : 2*		4 : 0 5 : 0
	0 : 7 0 : 7		1 : 1 3 : 6 6 : 11 1 : 4 7 : 4 2 : 1 2 : 4 7 : 10			
	0 : 8 0 : 8		4 : 3 3 : 7 2 : 4 3 : 4 3 : 2			
	0 : 8		Total 86 : 112			
	Total 0 : 61					
Murray Dark			17 : 11 10 : 8 4 : 2 5 : 3 14 : 10 11 : 8 2 : 4	15 : 4* 14 : 3* 10 : 4		18 : 0
			4 : 10 16 : 9 12 : 8 3 : 4 6 : 5 6 : 9 3 : 7 4 : 2	11 : 5 7 : 1* 20 : 4*		18 : 0
			Total 117 : 100	17 : 9 9 : 3* 6 : 2		
				11 : 3* 8 : 4 5 : 2 7 : 3		
				Total 140 : 47*		
Searls Dark			11 : 8 5 : 3 9 : 9 3 : 5 10 : 7 1 : 1 2 : 2 1 : 2	14 : 4* 14 : 5* 5 : 1		14 : 0 13 : 0
			11 : 9 6 : 6 2 : 6 5 : 4 5 : 5	19 : 8* 6 : 3 8 : 4 6 : 1*		12 : 0 18 : 0
			Total 71 : 67	5 : 2 Total 77 : 28*		26 : 0 7 : 0

All broods from red females are called 1 : 1 unless significantly different at the 5 per cent. level from that value. Broods from dark females are assigned to 3 : 1 when the exact probability of that ratio is greater than 50 per cent. and greater than the probability of a 1 : 1 ratio.

* Significantly different from 1 : 1 at the 5 per cent. level.

FIG. 4.—Map of the study area showing the percentage of red for 1967 at those sites where at least 100 isopods were collected.

a significant excess of red offspring. If differential viability or low penetrance were the cause, one would expect to see the effect in all four classes of females, but one does not. An alternative explanation is that a dominant red exists in the population at low frequency. If so, the excess red progeny would appear primarily in the broods from red females and some

3 red : 1 dark ratios would occur. Unfortunately, none of the young from Murray females has mated. Until more genetic evidence is available, one can only say that the hypothesis is plausible. In any case, the problem does not detract from the very clear evidence that in these populations red is usually recessive to dark.

5. FREQUENCY IN NATURAL POPULATIONS

During this study, 115 samples were collected at 38 sites. The most common frequency of red is about 10 per cent. with a range from 0 to 36 per cent. Since the variation in frequency shows no geographical pattern (figs. 3 and 4), the proportion of red must be site specific. Indeed, the frequency of red changes over a distance of only 20 metres at the Searls site (fig. 5). Overall, the 1967 samples from this site are homogeneous ($\chi^2_{(26)} = 35.32$, $P > 0.1$). However, the barn shows consistently lower frequencies, and a partition of the χ^2 shows that the difference between the barn and the remaining areas is highly significant ($\chi^2_{(1)} = 13.41$, $P < 0.001$). As the maps indicate, equally striking changes occur over distances of about one mile. When such abrupt shifts occur over short distances, one cannot expect to see a county-wide pattern or cline.

Several variables were recorded for each site: the nature of the cover, the type of vegetation, the amount of shade, the presence of other species of isopods, the nature of the soil, and the rate of drainage. No correlation was found between the frequency of red and any of these factors (Adamkewicz, unpublished). In addition, the colour forms were tested for fecundity, adult viability in the laboratory, and resistance to desiccation. No significant differences were found. The one monomorphic population occurred at a site that appeared in no way unusual. At the Searls site, the pen and barn were ecologically very similar while differing in the frequency of red. The pen and house differed in aspect, the nature of the cover, and vegetation; but they had the same frequency of red.

Nevertheless, two lines of evidence show that natural selection must be operating on the system. The first of these is the change of frequency with time. In Virginia, *A. nasatum* produces two broods per year between June and late August, and the population replaces itself every two or three years. The population size is over 6000 at the Searls pen site and probably that large at the Murray site. At the Murray site, the frequency of red was stable from September of 1964 to October of 1966, a span of at least four generations. The two other sites that were followed for over a year, Searls and Lewis Mountain, also had stable frequencies at all seasons. Sometime between October of 1966 and February of 1967 the frequency of red at the three sites dropped markedly and significantly (table 3). The low frequencies persisted throughout 1967, but by the spring of 1968 they had risen toward their former levels.

The study itself probably did not cause this decline. Collecting pressure was heavy throughout the year at the Searls and Murray sites; but the Lewis Mountain site was sampled only twice in 1966, both times well before the breeding season and twelve months before the 1967 collections. Furthermore, the Murray population had been sampled for three previous years without a change. Subjective changes in scoring procedures are not likely in view of this earlier consistency. These considerations suggest

that the study did not influence the polymorphism. Since the 1967 decrease took place during a period with no births, it must have been caused by a

FIG. 5.—Map of the Searls site, not to scale, showing the frequency of red and the location for some samples from 1967.

change in the relative death rates. Stability over several years followed by a rapid decline and a return toward normal certainly suggest that the polymorphism is a balanced one.

The second line of evidence shows that differential viability exists among the young. In table 4, the frequency of red is consistently, but not significantly, lower among males than among females. In 88 samples from

TABLE 3

*Analysis of the sampling data for the change of frequency over time*IA. *Collections from the Searls site*

Site and date	Number of samples	Red + Dark = Total	Frequency of red
House	5	271 + 1071 = 1342	0.2019
Pen	8	297 + 1170 = 1467	0.2024
House	5	180 + 1260 = 1440	0.1250
Pen	16	441 + 3242 = 3683	0.1197
Barn	6	84 + 940 = 1024	0.0820

IB. *Homogeneity χ^2 for Searls*

Class	Degrees of freedom	χ^2	Probability
1967	26	29.3959	n.s.
1966	12	11.4930	n.s.
Between	1	120.9875	less than 0.001
Total	39	161.8764	less than 0.001

IIA. *Collections from the Murray site*

Date	Number of samples	Red + Dark = Total	Frequency of Red
1964	2	154 + 311 = 465	0.3311
1965	4	240 + 411 = 651	0.3686
1966	8	545 + 980 = 1525	0.3573
1967	6	369 + 945 = 1314	0.2808

IIB. *Homogeneity χ^2 for Murray*

Class	Degrees of freedom	χ^2	Probability
1964	1	5.8294	less than 0.05*
1965	3	7.2416	n.s.
1966	7	8.5681	n.s.
1967	5	4.1383	n.s.
Among	3	23.6810	less than 0.001
Total	19	49.4584	less than 0.001

IIIA. *Collections from the Lewis Mountain site*

Date	Red + Dark = Total	Frequency of Red
October 1965	16 + 185 = 201	0.0796
March 1966	7 + 58 = 65	0.1076
April 1966	14 + 100 = 114	0.1228
March 1967	31 + 533 = 564	0.0549

IIIB. *Homogeneity χ^2 for Lewis Mountain*

Class	Degrees of freedom	χ^2	Probability
1965-1966	2	2.1461	n.s.
1967	0	—	—
Between	1	6.0762	less than 0.01
Total	3	8.2223	less than 0.05

* Involves a sample from an outlying area that was not available in later years.

17 sites, the frequency of red was higher in males than females in only 19 samples (for the deviation from 1 : 1, $\chi^2_{(1)} = 28$, $P < 0.001$). The observed sex ratio varies widely, but the frequency of red is lower among males no matter which sex is deficient. The evidence indicates that the variable sex ratio is caused by differential behaviour of the two sexes and that the underlying ratio is 1 : 1 (Adamkewicz, 1968). However, the variation prevents one from using the sampling data to determine which class is deficient, red males or dark females. Fortunately, the laboratory data show the same effect. At 7 months of age, the progeny of the wild-caught gravid females were 137 red males, 144 red females, 191 dark males and 144 dark females. Although the sex ratio overall and among reds is 1 : 1, it is not so among dark offspring ($\chi^2_{(1)} = 6.6$, $P < 0.01$). The deficiency is clearly in dark females.

6. DISCUSSION

The variation of body colour in *A. nasatum* is clearly a genetic polymorphism with a recessive gene resulting in the absence of a dark pigment and the unmasking of a light pigment that is always present. Preliminary work shows that black individuals of *A. vulgare* have the same two pigments. Although red animals of that species were not available, one can speculate that all recessive red phenotypes in the Oniscoidea have a similar basis. The nature of dominant red is unknown. The red morph of *A. nasatum* is dominant in France (Vandel, 1945), and a rare dominant may exist in Virginia. *A. vulgare* has both reds in a single population, and they are controlled by alleles, with dominant red the commoner of the two (Howard, 1953, 1962). Although *A. vulgare* occurs in Albemarle County, the red form has not been observed there.

The evidence does indicate that selection maintains the polymorphism. Only selection accounts satisfactorily for a polymorphism that exists in many populations at different, stable frequencies. Aside from one monomorphic population, the lowest observed frequency of red was 0.04, suggesting that random loss does not occur. Moreover, the change in frequency in 1967 and the consistently lower proportion in males both indicate differential mortality of the colour forms.

What the agent of selection may be is not clear, but winter weather was probably involved in the 1967 decline. The change coincided with unusually warm January temperatures, the highest in 11 years. The weather during January of 1968 was normal, and by April of 1968 the frequency of red had risen to a level intermediate between the 1966 and 1967 values. This observation strongly suggests that winter weather caused the original decline either directly through temperature effects or indirectly, perhaps by influencing the humidity.

The difference in phenotypic frequencies between the two sexes is rather unusual. Although the difference is not significant in any one sample, it is noticeable in nearly all. Either differential viability or sex influence on colour might account for this observation. If heterozygous females, but not males, were sometimes red, it would account for the inequality. However, red females should then be in excess among the progeny, and they are not. Furthermore, red males are scored "uncertain red" as often as females are. The more probable explanation is that dark females are less viable than dark males and that in natural populations this leads to a higher frequency

TABLE 4
Frequency of red for each sex in single samples from various sites

Date	Site	Number of		Freq. Red males	Number of		Freq. Red females	S.E.
		Red males	Dark males		Red females	Dark females		
3/65	Murray	105	207	0.337	112	171	0.365	0.017
6/65	Lambeth	1	21	0.045	14	201	0.062	0.014
11/65	Red Hills	10	77	0.115	20	100	0.145	0.022
4/66	Hastings	7	79	0.081	17	97	0.120	0.022
6/56	Searls	35	158	0.181	32	103	0.204	0.020
11/66	Dairy Queen	10	135	0.069	21	146	0.099	0.014
3/67	Lewis Mountain	11	185	0.056	14	228	0.057	0.010
4/67	Airport Motel	13	81	0.138	17	86	0.152	0.024
4/67	Farm	11	68	0.139	27	116	0.171	0.024
6/67	Makielski	—	50	0.000	—	86	0.000	—
7/67	Advance Mills	1	91	0.011	10	141	0.045	0.010
7/67	Earlsville	7	49	0.125	6	84	0.089	0.022
7/67	Hacienda	4	32	0.111	17	53	0.198	0.037
7/67	Knight	—	54	0.000	6	61	0.049	0.017

of red among females. Since the frequency of red in males is the same among first- and second-year animals, the deficiency must be established before the secondary sex characters develop. Most young reach sexual maturity in April or May at the age of about 8 months, suggesting that differential mortality, like the unusual change in 1967, occurs during the winter months.

The results in tables 1 and 2 indicate that homozygous dark animals of both sexes are deficient in natural populations. The frequency of non-segregating dark broods from red females should equal the frequency of homozygous dark males in the population, yet only two such broods appear in table 2. Non-segregating broods from dark females are also deficient in both tables. Even the presence of a rare dominant red cannot account for this latter deficiency.

The evidence does favour a real deficiency of dark females as the cause of different phenotypic frequencies in the two sexes. Instances of sex-limited polymorphisms are common in mimetic butterflies (Ford, 1964), and the polymorphism for pattern in *A. vulgare* is sex limited to females (Howard, 1962). However, cases are very rare in which the sexes differ in phenotypic frequencies in the absence of sex linkage or sex limitation. Spotting in the butterfly *Maniola jurtina* may be an example (Dowdeswell, 1961), but sex influence on the phenotype is an equally likely interpretation of this case. In contrast to its rarity in other groups, the phenomenon may be common in oniscoid isopods. Although he does not comment on it, Howard's collections (1940, 1962) show that the frequency of red is lower in males than females of *A. vulgare*, and de Lattin (1951, 1954) has shown the same for marbled body colour in *Porcellio scaber*. The genes in *A. vulgare* and *P. scaber* are dominant; in *A. nasatum*, recessive. However, a single locus controls each character, and none is sex limited.

One model of selection can account for all three polymorphisms. If selection acts primarily or entirely against one homozygote in one sex and the other homozygote in the other sex, the result is a special case of heterozygous advantage. One result is the deficiency of one phenotype in each sex. The existence of a truly sex-limited polymorphism in *A. vulgare* is evidence that the selective advantages of colour and pattern can differ in the two sexes of isopods.

A. nasatum and *P. scaber* show deficiencies in the laboratory as well as in natural populations. Cultures of *P. scaber* breeding true for marbled contained about 32 per cent. males while cultures of solid coloured animals were about 69 per cent. male. In the combined brood data for *A. nasatum*, red progeny were 49 per cent. male, and darks were 57 per cent. male. De Lattin prefers the hypothesis that the pattern locus influences sex determination, but he notes that differential viability might also explain the data. In natural populations of *P. scaber*, the frequency of males among marbled increases with increasing overall frequency of marbled (de Lattin, 1951, 1954). If colour affects sex determination, the observation requires an intricate explanation, while it is exactly what one expects if different viabilities in the sexes maintain the polymorphism.

7. SUMMARY

1. *A. nasatum* in Albemarle County, Virginia, possesses three colour forms, red, black, and brown. At least two, red and black, are genetically

distinct. A recessive gene controls red, but some evidence suggests that a dominant gene for red is also present.

2. The body colours are due to ommochrome pigments in the epidermis. Dark isopods have two pigments only one of which is present in red animals.

3. The frequency of red in natural populations varies over both space and time. The typical value in Albemarle County is about 10 per cent. with extremes of 0 and 36 per cent. The shortest distance over which a change in frequency was detected was approximately 20 metres; the shortest time, four months.

4. The frequency of red declined markedly in widely separated populations during the winter of 1966-67 and rose again in the spring of 1968. The probable cause was unusually warm weather in January of 1967.

5. No correlation was found between the frequency of red and any environmental variable.

6. In samples from natural populations the frequency of red is lower among males than among females. Dark females are deficient in laboratory raised broods, accounting for the lower frequency in males. This difference is established before sexual maturity. Homozygous dark adults of both sexes may be deficient. Selection primarily against both dark females and red males could provide conditions sufficient to maintain a balanced polymorphism.

Acknowledgments.—The author gratefully acknowledges the help of Dr J. J. Murray, Jr., who first drew her attention to the polymorphism and whose constant guidance and advice made the study possible. I thank the many people who permitted the use of their land for their kindness and co-operation. I also thank Professor Dietrich Bodenstein for providing the facilities for this study.

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