

## THE GENETICS OF ARMADILLIDIUM VULGARE LATR.

## IV. LINES BREEDING TRUE FOR AMPHOGENY AND THELYGENY

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## I. INTRODUCTION

One of the interesting genetical features of woodlice, terrestrial Isopoda, is that in many species, in addition to females (amphogenics) which produce broods with a 1 : 1 sex ratio, there also occur other females (thelygenics) which produce broods consisting nearly entirely of females, and sometimes still other females (arrhenogenics) which produce broods containing mostly males (Vandel, 1941). In the woodlouse, *Armadillidium vulgare* Latr., it was found (Howard, 1942, Table 15) that of 22 females collected from wild populations near Cambridge 11 were amphogenics, 9 were thelygenics and 2 were peculiar animals, one of them producing a more or less thelygenic brood in one year and an arrhenogenic brood in the following year and the other producing an amphogenic brood in the first year and a thelygenic brood in the second year.

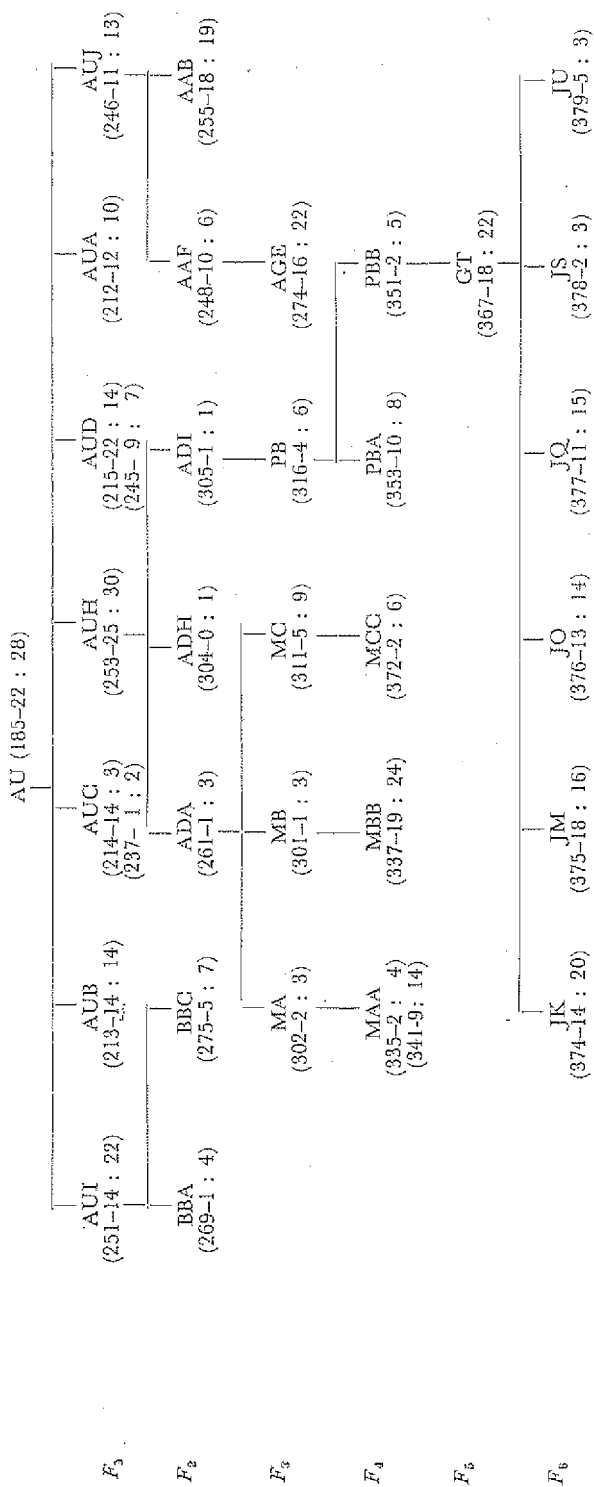
Studies on the inheritance of amphogeny, thelygeny and arrhenogeny in *Armadillidium vulgare* by Howard (1942) did not suggest any simple scheme. It was found that one thelygenic female gave thelygenic, amphogenic and arrhenogenic daughters, that two other thelygenic females gave mostly thelygenic daughters, that one arrhenogenic female gave thelygenic, amphogenic and arrhenogenic daughters, that one amphogenic female gave thelygenic and arrhenogenic daughters, and finally that one amphogenic female gave only amphogenic daughters.

The results given in the present paper are simple. All the descendants of one amphogenic female have been amphogenics, and all but one of the descendants of one thelygenic female have been thelygenics. It must be emphasised that the regularity of these results has not been brought about by conscious selection for regularity of behaviour of sex ratios. On the contrary the animals bred from had been used for studies on the inheritance of recessive red body colour (Howard, 1953), and only later was it noticed that the sex-ratio results were interesting. One generation (the  $F_4$ ) in the thelygenic line and three generations ( $F_4$ ,  $F_5$  and  $F_6$ ) in the amphogenic line were, however, obtained for studies of sex ratios.

## II. FEMALES AU AND GN

Both females AU and GN were collected from the base of the same elm tree near the roadside at Four Went Ways, Abington, Cambridge. Both females were pregnant when collected, and thus their broods show sex ratios that would presumably have occurred

Table 1. Amphitogenic line—descendants of female AU



The figures in brackets underneath the letters denoting the female are first the number of the brood and secondly the sex-ratio, in each case the number of males being given first and the number of females second—thus AUI (251-14 : 22) signifies that female AUI produced brood 251 in which there was a sex ratio of 14 males : 22 females.

under natural conditions. Female AU, collected on May 28th, 1944, was red, type B; and female GN, collected on July 11th, 1945, was red, type D (for type B and sex-limited type D, see Howard, 1940). The red body colour of both animals, as opposed to the more usual black or grey, was due to a recessive factor (Howard, 1953). Brood 185 from female AU contained 22 males and 28 females, and there was a segregation for body colour of 15 red; 35 black, suggesting that female AU had mated with a black male heterozygous for recessive red. Brood 233 from female GN contained 3 males and 92 females. All animals were black, but there was a segregation in the females of 48 type B: 44 type D. Female GN had therefore mated with a black male homozygous for black and not carrying the dominant factor for the sex-limited type D.

### III. AMPHOGENIC LINE—DESCENDANTS OF FEMALE AU

Results for the amphogenic line, descendants of female AU, are given in Tables 1 and 2. Unfortunately many of the broods were small. This small size of broods was probably due to a number of causes. First the animals were scored for red v. black at an early stage before scoring for sex is easy, and this early scoring can lead to the death of animals which are about to or have just moulted. Secondly there appeared at times to be some

Table 2. Summary of male : female segregations for amphogenic line descended from female AU (see Table 1)

Generation of females	Segregations males : females	$\chi^2$ for difference from 1 : 1	P
P	22 : 28	0.720	0.40
F <sub>1</sub>	122 : 115	0.207	0.65
F <sub>2</sub>	36 : 41	0.325	0.57
F <sub>3</sub>	28 : 43	3.169	0.075
F <sub>4</sub>	44 : 61	2.752	0.097
F <sub>5</sub>	18 : 22	0.400	0.53
F <sub>6</sub>	63 : 71	0.478	0.49
Total	343 : 381	1.994	0.16

Heterogeneity  $\chi^2=6.057$ ;  $n=6$ ,  $P=0.42$

disease which killed many young animals. Thirdly many animals were bred from in their first year when they were rather small. Fourthly there may also have been some depression in vigour due to inbreeding. On the whole, however, the results certainly suggest that the line is breeding true for amphogeny (i.e., 1 : 1 sex-ratios).

It can be seen from the figures given in Table 2 that the total segregation for males v. females in this line shows a small, but not significant, excess of females. Similar small excesses of females have also been found for amphogenics (or supposed amphogenics) in *Armadillidium vulgare* by Howard (1942) and in other woodlice, see Table 3. The only

Table 3. *Sex ratios in broods from amphogenic females of Armadillidium vulgare and other woodlice*

Species	Reference	Sex ratio males: females	% females	$\chi^2$	<i>P</i>
<i>Armadillidium vulgare</i>	This paper, Table 2	343 : 381	52.7	1.99	0.16
" "	Howard (1942, Table 11)	185 : 201	52.1	0.66	0.41
" "	Howard (1942, Table 15)	385 : 459	54.5	6.49	0.011
<i>Trichoniscus provisorius</i>	Vandel (1938)	1,565 : 1,647	51.3	2.09	0.15
<i>Oniscus asellus</i>	Vandel (1941)	157 : 176	52.8	1.08	0.30
<i>Armadillidium nasatum</i>	Vandel (1941)	1,016 : 1,178	53.7	11.96	0.00054
Total		3,651 : 4,042	52.5	19.87	0.00001
Total (omitting Howard, 1942, Table 15 and Vandel, 1941 on <i>Armadillidium</i> )		2,250 : 2,405	51.7	5.16	0.023

cases in Table 3 where this excess of females is significant are those of Howard (1942), Table 15, and Vandel (1941). The excess of females in the total for all the other cases is, however, significant. This is slight evidence for the female being the heterogametic sex in woodlice.

#### IV. THELYGENIC LINE—DESCENDANTS OF FEMALE GN

Results for the line breeding true for thelygeny are shown in Table 4 and summarised in Table 5. On the whole the broods are considerably larger than those in the amphogenic line (this may be due to there being no inbreeding which was usually impossible because there were few or no males in the broods). There is one brood, brood 336 from female QAC in the  $F_4$ , which is not thelygenic but which is arrhenogenic (39 males : 7 females). Apart from this brood and possibly also brood 271 from female AHA in the  $F_2$  (3 males : 6 females), all the other broods would appear to be thelygenic. Thus thelygeny has persisted for four generations, despite the fact that in nearly every case the females were mated with males from non-thelygenic broods.

#### V. SEX DETERMINATION IN WOODLICE

Vandel (1938) based his explanation of monogenic (i.e., thelygenic and arrhenogenic) broods in the woodlouse *Trichoniscus provisorius* on the hypothesis that in woodlice the female is the heterogametic sex and that there was in thelygenic and arrhenogenic females directed segregation of the  $\dot{X}$  and  $X$  chromosomes respectively into the eggs. Vandel (1947) was, however, unable to obtain any cytological evidence for sex chromosomes in woodlice. Sex chromosomes, the female being the heterogametic sex, have, on the other hand, been found in one species of the class Isopoda, in the marine species *Jaera marina*, by Staiger and Bocquet (1954).

In a later paper Vandel (1941) came to the conclusion that "la fréquence de l'hermaphroditisme chez les représentants les plus divers de l'ordre des Isopodes, et la variabilité extrême de la répartition des sexes, inclinent à penser que la détermination du sexe est d'ordre phénotypique, plutôt que génétique, chez ces animaux." De Lattin (1949, 1951 & 1952), while not supporting a phenotypic determination of sex, has suggested



Table 5. *Summary of male: female segregations for the thelygenic line, descendants of female GN (see Table 4)*

Generation of females	Segregations males: females
<i>P</i>	3 : 92
<i>F</i> <sub>1</sub>	4 : 41
<i>F</i> <sub>2</sub>	5 : 46
<i>F</i> <sub>3</sub>	8 : 145
<i>F</i> <sub>4</sub> (omitting brood 336)	14 : 516
<i>F</i> <sub>5</sub>	0 : 12
Total	34: 852

that in woodlice there is a multifactorial determination of sex, i.e., a number of factors in several chromosomes (see also section VII of this paper). Thus he claims to have shown that in some species an autosomal dominant gene for a colour pattern has the effect of producing more females in the broods from animals carrying such factors.

It would appear, however, from the fact that true amphogenic females exist in at least some species (e.g., the line descended from female AU described earlier) that, although sex chromosomes cannot be recognised cytologically, sex determination is by an orthodox single switch gene or group of closely linked genes in one pair of chromosomes as occurs in animals where an *XY* chromosome mechanism can be recognised by cytological or genetical means.

Although intersexes do occur in natural woodlice populations, they are usually rare. Thus their frequency in the population from which females AU and GN were obtained was less than 1 in 1,000. Two of these intersexes, which were recognised in the first place by their having an intermediate type of colour between type A (the normal male colour) and types C and D (sex-limited to females) and by the male styles being very short, have been further examined by Collinge (1946; 1947). No intersexes have been found in the many broods bred in the laboratory. It would thus appear that intersexes are very rare, and not fairly common as might be expected on either a phenotypic or multifactorial scheme for sex determination. In some populations intersexes have been found in larger numbers, but they would in most cases appear to be males parasitically castrated by the Tachinid fly *Phyto melanocephala* (Thompson, 1934).

## VI. THELYGENIC AND ARRHENOGENIC BROODS

Brood sizes in the present investigation were much too variable for showing that monogenic broods are as large as amphogenic broods, but previous work (Howard, 1942) showed that in *Armadillidium vulgare* both types of brood contained about the same numbers of animals. Also Vandell (1938, 1941) and de Lattin (1952) have found for other species of woodlice that monogenic (both arrhenogenic and thelygenic) and amphogenic broods are of similar size. The absence of males in thelygenic broods and that of females

in arrhenogenic broods is not due therefore to the non-viability of one sex as has, for example, been found in *Drosophila bifasciata* by Magni (1953).

Parthenogenesis also cannot in *Armadillidium vulgare* account for the different types of broods. By using males carrying the dominant factor for red body colour, Howard (1942) showed that fertilization took place in both monogenic and amphogenic broods. It was also found (Howard, 1942) that segregation for red body colour in the eggs was normal in both monogenic and amphogenic broods.

Finally it has been found by Vandel (1938, 1941), by Howard (1942) and by de Lattin (1952) that the male has no influence on the sex-ratio of a brood. The male may, however, according to de Lattin (1952), have an influence on the proportions of amphogenic and monogenic daughters produced by amphogenic females, and on the proportion of thelygenic and arrhenogenic daughters produced by thelygenic females (see later, Section VII-B and Table 6).

## VII. INHERITANCE OF AMPHOGENY AND MONOGENY

### A. *Trichoniscus provisorius*

Vandel (1938) distinguished in *Trichoniscus provisorius* six types of females—(a) amphogenics, (b) perfect thelygenics, (c) imperfect thelygenics, (d) perfect arrhenogenics, (e) imperfect arrhenogenics, (f) allelogenics and (g) mixed females. It should, however, be noted that broods are small in this woodlouse, and that Vandel did not apply statistical criteria to his results. His distinctions between perfect and imperfect thelygenics (the latter give broods with occasional males) and between perfect and imperfect arrhenogenics are therefore not necessarily valid. Allelogenic females are animals which give thelygenic and arrhenogenic broods at different times. Mixed females are animals, born as members of a thelygenic brood, but which themselves give amphogenic broods and whose daughters may again be thelygenics. On the whole Vandel found that amphogenics produced amphogenic daughters, and that thelygenics had either thelygenic or arrhenogenic daughters. Also the exceptional females in imperfect arrhenogenic broods were themselves arrhenogenics.

Vandel explained the above results by suggesting that monogeny was determined by cytoplasmic inheritance (inheritance by factors on the *Y* chromosome would give similar results, see Howard, 1942). Vandel's explanation, however, does not account for the difference between thelygenic and arrhenogenic females and as to why thelygenic females produce about 50% of arrhenogenic daughters. The relatively rare mixed females are on the other hand not unexpected since many cytoplasmically determined conditions (e.g., male sterility in maize, Rhoades, 1933) show similar irregularities. The allelogenics are interesting in showing that the balance between thelygeny and arrhenogeny is easily altered. While no explanation for the origin of arrhenogenic daughters from thelygenic mothers can be given, it would appear reasonable to suggest that, if monogeny is caused by a cytoplasmic condition influencing the segregation of the *X* and *Y* chromosomes into the eggs or polar bodies, then this cytoplasmic condition can be altered in certain cases by chromosomal genes and perhaps in other cases by environmental factors.

Table 6. *Summary of main results obtained by de Lattin (1952) for*  
*Cylisticus convexus*

Female	Parents		Daughters		Monogenics
		Male from	Amphogenic : Monogenic		Thelygenics : Arrhenogenics
Amphogenic	Amphogenic brood		396 : 97		46 : 51
Amphogenic	Arrhenogenic brood		101 : 96		62 : 34
Thelygenic	Arrhenogenic brood		163 : 1016		514 : 502
Thelygenic	Amphogenic brood		55 : 391		270 : 121

B. *Cylisticus convexus*

The main results of de Lattin (1952) for *Cylisticus convexus* are summarized in Table 6 of this paper. They are particularly interesting in showing that the male parent has some influence on the proportions of the different types of daughters produced. Thus in the progeny of amphogenic females, a father from an amphogenic brood gives about 20% of monogenics while a father from an arrhenogenic brood gives about 50% of monogenics. Also in the progeny of thelygenic females, while the proportions of monogenic and amphogenic daughters do not differ with the two types of father, there would appear to be in the monogenic daughters equal numbers of thelygenics and arrhenogenics when the father came from an arrhenogenic brood, but 2 thelygenics : 1 arrhenogenics when the father came from an amphogenic brood (Table 6 of this paper).

It should also be noted (Table 6) that amphogenic females  $\times$  males from amphogenic broods give mainly amphogenic daughters, and that thelygenic females  $\times$  males from arrhenogenic broods give mainly monogenic daughters which are about equally divided into thelygenics and arrhenogenics. There could thus be two types of *Cylisticus convexus* populations which would have equilibrium conditions for sex-ratios, one consisting of amphogenics only and the other containing only monogenic females, one half of these being thelygenics and the other half being arrhenogenics (compare Table 13 in Howard, 1942).

De Lattin's (1952) explanations of his results for *Cylisticus convexus* are somewhat complicated, and they have been severely criticised by Goldschmidt (1956, pages 466-69). First de Lattin suggests that sex-determination is polyfactorial by  $M$  and  $F$  complexes spread over the autosomes, the  $M$  and  $F$  complexes being more or less equal in their effects and all animals being heterozygous. Secondly a dominant factor  $F'$  is postulated—this factor is one of the realizers of the  $F$  complex and has a predetermining feminizing effect through a substance  $\phi$  in the cytoplasm. Thirdly there is another factor,  $I$ , which increases the feminizing effect of  $F'$  so that more  $\phi$  substance is produced. On this scheme thelygenic females are  $F'fII$  (or  $F'fIi$ ) and, when mated with males from arrhenogenic broods which have the constitution  $ffII$  (or  $ffIi$ ), they produce two types of offspring,  $F'fII$  and  $ffII$ . Both are females because of the predetermining feminizing effect of  $F'$  and  $I$  together through the substance  $\phi$ . The  $F'fIi$  females will be thelygenics like their mother, but the  $ffII$  will be arrhenogenics. This is an ingenious explanation to account for the production by thelygenic mothers of 50% of thelygenic daughters



and 50% of arrhenogenic daughters, but it should be noted that to give a full explanation of all the results for *Cylisticus convexus*, e.g., the occurrence of amphogenic daughters in the offspring of thelygenic females, further hypotheses have to be made.

### C. *Armadillidium vulgare*

The results for *Armadillidium vulgare* given in sections III and IV of this paper would support the original hypothesis of Vandel (1938), i.e., monogeny is inherited as if due to cytoplasmic factors which control the segregation of the *X* and *Y* chromosomes (alternatively it could be due to factors on the *Y* chromosome). This hypothesis, as we have noted earlier, does not, however, account for the two types of monogeny nor as to how arrhenogenic daughters are produced from thelygenic females. Neither will the original Vandel hypothesis explain some of the irregular results recorded by Howard (1942). A possible explanation of such results both in *Armadillidium vulgare* and in other woodlice might be on the basis of *X* and *Y* chromosomes of different strengths for segregation behaviour. There is, however, as has been pointed out earlier, no demonstration yet that an *XY* sex chromosome mechanism does exist in woodlice.

## VIII. SUMMARY

1. Sex ratios of broods were studied in two lines of the woodlouse, *Armadillidium vulgare*. The two lines originated from two females, pregnant when collected from a wild population.
2. One line was found to breed true for amphogeny (1 : 1 sex ratios), and the other line bred almost true for thelygeny (nearly 100% females).
3. In the amphogenic line there was a small excess of females over males. Similar excesses of females are found in broods from amphogenic females of other woodlouse species.
4. Sex determination in woodlice, the origin of monogenic broods, and the inheritance of monogeny are discussed.

## REFERENCES

- COLLINGE, W. E. (1946). Hermaphroditism in a terrestrial Isopod. *Nature, Lond.*, **157**, 304.
- COLLINGE, W. E. (1947). Further notes on the terrestrial Isopoda. *Northwestern Naturalist*, **22**, 84—87.
- GOLDSCHMIDT, R. (1956). Theoretical Genetics. University of California Press, pp. 563.
- HOWARD, H. W. (1940). The genetics of *Armadillidium vulgare* Latr. I. A general survey of the problems. *J. Genet.*, **40**, 83—108.
- HOWARD, H. W. (1942). The genetics of *Armadillidium vulgare* Latr. II. Studies on the inheritance of monogeny and amphogeny. *J. Genet.*, **44**, 143—59.
- HOWARD, H. W. (1953). The genetics of *Armadillidium vulgare* Latr. III. Dominant and recessive genes for red body colour. *J. Genet.*, **51**, 259—69.
- LATTIN, G. DE (1949). Ein Farbgen als relativer Geschlechts-realisator bei *Porcellio scaber* (Isopoda). *Naturwiss.*, **36**, 89.

- LATTIN, G. DE (1951). Über die Bestimmung und Vererbung des Geschlechts einiger Oniscoideen (Crust., Isopod). I. Mitt. Untersuchungen über die geschlechts-beeinflussende Wirkung von Farbfaktoren bei *Porcellio* und *Tricheoniscus*. *Z. indukt. Abstamm. u. Vererblehre*, **84**, 1-37.
- LATTIN, G. DE (1952). Über die Bestimmung und Vererbung des Geschlechts einiger Oniscoideen (Crust., Isopod). II. Mitt. Zur Vererbung der Monogenie von *Cylisticus convexus* (Deg.). *Z. indukt. Abstamm. u. Vererblehre*, **84**, 536-67.
- MAGNI, G. E. (1953). 'Sex-ratio', a non-Mendelian character in *Drosophila bifasciata*. *Nature, Lond.*, **172**, 81.
- RHOADES, M. M. (1933). The cytoplasmic inheritance of male sterility in *Zea Mays*. *J. Genet.*, **27**, 71-93.
- STAIGER, H. & BOCQUET, C. (1954). Cytological demonstration of female heterogamety in Isopods. *Experientia*, **10**, 64-66.
- THOMPSON, W. R. (1934). The tachinid parasites of woodlice. *Parasitology*, **26**, 378-448.
- VANDEL, A. (1938). Recherches sur la sexualité des Isopodes. III. Le déterminisme du sexe et de la monogénie chez *Trichoniscus* (*Spiloniscus*) *provisorius* Racovitza. *Bull. biol.*, **72**, 147-86.
- VANDEL, A. (1941). Recherches sur la génétique et la sexualité des Isopodes terrestres. VI. Les phénomènes de monogénie chez les Oniscoïdes. *Bull. biol.*, **75**, 316-63.
- VANDEL, A. (1947). Recherches sur la génétique et la sexualité des Isopodes terrestres. X. Étude des garnitures chromosomiques de quelques espèces d'Isopodes marins, dulcaquicoles et terrestres. *Bull. biol.*, **81**, 154-77.