This paper reports sex ratios in individual sibships and in adult populations, the effects of inbreeding and the likely interaction of these features on fitness in the isopod, Venezillo evergladensis. Sex determination in terrestrial isopods has proved difficult to explain. The large number and small size of the chromosomes (2N = 54 in Armadillidium nasatum) have prevented recognition of specific sex chromosomes (Teichmann, 1962). Some workers have argued for heterogametic females, still others for heterogametic males though all arguments have been based on indirect evidence. Much of the evidence is reviewed by Bacci (1965). The isopod literature recognizes three female types, amphogenics producing broods with 1:1 sex ratios, thelygenics, and arrhenogenics producing broods with higher proportions of females and males respectively. The term, monogeny, simply denotes the presence of either of the latter two female types.

The cause of monogeny in the progenies of different females may well provide an insight to the relative fitness between amphogenic and monogenic females. The first serious attempt to identify the causative mechanism dates back to Vandel (1938). Judging from a series of breeding studies in France on the genera Trichoniscus and Armadillidium, Vandel (1938, 1939) explained monogeny as a response to a cytoplasmic factor transmitted by females. The factor's presence could presumably override the nuclear role of sex determination. Howard (1940, 1942, 1958) continued inquiries by breeding studies using Armadillidium vulgare. He obtained lines breeding true for amphogeny and thelygeny and found evidence for a maternal influence, though a paternal effect on the daughter's type could not be excluded. He also found sibship size between female types did not vary in a meaningful way and was unable to explain the absence of one sex by differential mortality prior to scoring. More recently, Juchault and Legrand (1970, 1972) and Legrand and Juchault (1972) provided convincing evidence for cytoplasmic factors having self reproducing, infective properties that act to modify the sex ratios. These investigators experimentally converted females from amphogeny to thelygeny, and the case is similar to the spirochete-like particle affecting sex ratios in Drosophila willistoni (Poulson and Sackguchi, 1961). Amphogenic females are free of the particles. It is not clear whether thelygenics and arrhenogenics reflect different particles or a genetically different response to the same particle. Laboratory breeding data reveal high frequencies of monogenic females and thus indirectly attest to high frequencies of such females in the populations providing the breeding stocks. Such data for V. evergladensis are given below. Samples of females taken directly from nature have infrequently been scored by the sex ratios of their offspring. Vandel (1938, 1939) found 41 arrhenogenics and 32 thelygenics in a sample of 88 females of Trichoniscus provisorius, and four each of these two types in 15 females of Armadillidium vulgare. Nine of 30 gravid females of the latter species I collected in Gainesville, Florida, produced broods, exceeding 68 young per brood, departing from 1:1 ratios at the .05 probability level, 4 and 5 having greater female and male numbers respectively.

Thus, monogeny occurs in a frequency capable of playing a significant role in
natural selection. Howard (1942) suggested monogeny’s advantage lay in its reduction of inbreeding. Howard’s experience with his laboratory colony, providing genetic data over a number of years, must have suggested an inbreeding depression; however, he presented no evidence to this effect. This paper provides an analysis of inbreeding and also directs attention to an additional problem resulting from monogeny, namely, evolution of sex ratios along lines suggested by R. A. Fisher (1958).

MATERIALS AND METHODS

Venezillo evergladensis is an armadillid species occurring in Georgia and Florida and generally similar in structure and ecology to its more familiar relative Armadillidium vulgare. The species is particularly adaptable to laboratory culture. Data for this study were accumulated from pair matings in the genetic analysis of the red body r locus (Johnson, 1976). That paper outlines in detail procedures for maintaining stocks, setting-up pair matings and recording brood data. Briefly, individuals mature sexually in 60 to 80 days, females may engage in multiple matings, may store viable sperm well over a year, and the young are produced in successive but distinct broods. Since survival of individuals in my laboratory colony probably exceeds life expectancies in the wild, the observed performances in fecundity most likely include the biologically meaningful period.

The birth of each brood per pair was recorded after no more than four days of age; the majority of broods were probably noted one to two days after release from the brood pouch. The interval between broods is thus a small overestimate and begins between broods one and two, because no time can be set for initial mating after setting up a cross. Each brood was counted on transfer to a separate container within 36 hours of first observation giving an early value of brood size. I observed no cannibalism or other parent associated decrease in survival prior to recording brood size. Thus, brood size, judged as exact counts, and intervals between successive broods, judged as small over estimates randomized over all broods, are available.

Stocks for genetic studies originated from broods of field collected females from the Compost Population, Gainesville. Crosses between these individuals and between their subsequent offspring were made relative to the r locus and two other loci currently under study and were not designed for inbreeding data. Pedigrees exist, however, for each isopod back to its original field collected female ancestor, and the crosses include numerous combinations producing inbred broods. Excepting one category of crosses, inbreeding coefficients, \((F)\) have been computed from pedigree pathways giving samples having \(F\) values of 0, .125, .250 and .375. The field collected females are assumed to have the same degree of relationship, and their \(F\) values are taken as zero to expedite computation of \(F\) values among their progeny. The field collected females are known to engage in multiple matings (Johnson, 1976) and their offspring may be full or half-sibs. Matings between offspring of these females may thus be full or half-sib inbreeding. Consequently, broods from lineages started from matings between sibs of field collected females have not contributed data. Inbred and non-inbred broods may be compared for mean brood size, change of brood size with successive brood number, mean interval between broods and change of interval between successive broods. A total of 172 crosses contributed data. For brood size, 75, 16, 53 and 28 crosses exist with \(F\) values of 0, .125, .250 and .375 respectively, and for intervals between broods, 60, 0, 37 and 20 crosses contributed data for \(F\) values in the same order. Broods having an \(F\) of .125 arrived late in the breeding studies after the routine of checking stocks was changed. Thus brood size for these crosses are suitable values but intervals between broods are not comparable to other interval data. Sex ratio values exist for 87 sibships. Sex is scored by presence or absence of the male pleopods at approxi-
EVOLUTION OF ISOPOD SEX RATIOS

approximately 40 days of age. Survival is typically near 100 percent under the laboratory conditions and a knowledge of brood size at birth clearly dismisses the possibility of differential mortality on sexes leading to unequal ratios.

Monogeny can only reduce full or half-sib matings \( (F = .25 \text{ or } .125 \text{ respectively}) \). First-cousin matings \( (F = .125) \) are not affected and presumably second generation sib matings \( (F = .375) \) decrease with reduction of such first generation matings. The long life of individuals relative to development time introduces the possibility of parent-offspring matings; however, the presence or absence of monogeny in the parent’s broods cannot reduce this form of inbreeding. Thus, the meaningful comparison bearing on monogeny consists of full and half-sib matings with those of non-inbreds. Also, the sex ratio of the full sibship rather than of separate broods is the important value as individuals seem willing to mate with all mature ages.

**Sibship Sex Ratios**

Sex ratios were taken on the 87 sibships prior to computing their \( F \) values and the distribution took the following form.

\[
\begin{align*}
F = 0 & \quad 45 \text{ crosses} \quad \text{Sibship sizes, 13–68.} \\
F = .125 & \quad 2 \text{ crosses} \quad \text{Sibship sizes, 16, 18.} \\
F = .250 & \quad 30 \text{ crosses} \quad \text{Sibship sizes, 13–66.} \\
F = .375 & \quad 10 \text{ crosses} \quad \text{Sibship sizes, 11–43.}
\end{align*}
\]

Chi-square tests were performed on each family comparing observed male : female numbers to expected 1 : 1 values for the sibship size. Eleven of the 45 crosses with an \( F \) of zero departed from 1 : 1 values, 4 and 7 at probability < .05 and < .01 respectively. Six and five of these families possessed greater female and male proportions respectively. Likewise, nine of the 30 crosses with an \( F \) of .250 departed from 1 : 1 values, 5 and 4 at \( P \) values < .05 and < .01 respectively, also 5 and 4 families possessed greater female and male proportions respectively. Only one of the ten crosses with an \( F \) of .375 departed from 1 : 1 values at a \( P < .01 \) and possessed a high male proportion. Both crosses with an \( F \) of .125 were consistent with 1 : 1 values.

No heterogeneity appears to exist between \( F \) values and unequal ratios. For the total sample, 21 of 87 sibships or 24.1 percent departed too much from 1 : 1 values to be explained by chance. For the 45 families having an \( F \) of zero and for families with an \( F \) of .250, 24.4 and 30 percent respectively were significantly departing from a 1 : 1 ratio. Nine of the 21 unequal ratios, while having low likelihoods, may not be overly impressive. The ratios of observed female number to total family size for these nine crosses are 27/41, 20/27, 30/45, 3/14, 4/17, 16/22, 6/21, 13/16 and 7/25. The remaining 12 families with probabilities < .01 for 1 : 1 ratios had females to total numbers as follows: 1/19, 2/45, 1/57, 1/15, 5/25, 13/48, 45/66, 28/37, 30/42, 26/36, 40/58 and 32/32. Clearly these ratios depart meaningfully from 1 : 1 values and constitute 13.8 percent of the total sample of 87 sibships. If natural selection distinguishes between females producing families with different sex ratios, its operation by-way-of the sex ratios of *Venezillo evergladensis* is near certainty.

**Inbreeding Effects**

The most direct comparison of relative fecundities concerns the production of offspring number. The young are produced in discrete broods and, as shown below, brood size increases with successive brood number. Consequently, comparing fecundities by mean brood size requires mean values for the same group of successive broods. Eight successive broods exist for a number of crosses though most families involve fewer numbers. The mean size of the first three successive broods was selected for comparison since the group having an \( F \) of .125 dropped below 30 if larger brood numbers are used. Sexual maturity develops in 60 to 80 days, and three broods following this period require about 90 additional days, as shown below. The size of individuals after five months of age...
approximates the maximal size observed in nature. Thus, five months is a reasonable estimate of longevity in nature. A comparison of the first three broods has biological meaning in any event; however, if the age estimate is correct, later unmeasured and possibly compensating parameters are unimportant. The mean brood sizes of the first three broods for the four \( F \) values are given in Table 1. The \( t \)-test shows fecundity of nonrelated parents exceeds that of first and second generation full-sib matings (\( F \) of .250 and .375, at \( P \) values of < .01 and < .02 respectively). The performance of half-sibs and first-cousin matings (\( F \) = .125) is lower than for nonrelated parents but not significantly, and also does not differ significantly from the full-sib values. If families consist of male and female full-sibs and such individuals mate, then the future representation of their parents' genetic material is less than if their progeny had not inbred. A shift to monogenic females could reduce this loss of representation. Multiple mating leads to families including male and female half-sibs. Mating between these half-sibs shows no significant loss of parental representation and a shift to monogeny offers no advantage. The lower mean for half-sib matings is suggestive of a lower fecundity, and more data for such matings are desirable. Regression coefficients for brood size on brood order number where the first and second successive broods are numbers 1, 2, etc. also appear in Table 1. All broods for all crosses contribute data to this analysis. All coefficients are positive and significantly greater than zero at \( P < .001 \) excepting the \( F \) of .125 group where \( P < .02 \). Thus brood size increases with successive broods, and the coefficient for nonrelated parents exceeds the full-sib coefficient at \( P < .02 \). Significant differences are lacking between other coefficient pairs. Again, monogeny could improve subsequent fecundity if families are largely full-sibs and sib matings are frequent. Half-sib matings lead to no significant loss in their parents' future representation.

The mean day interval between broods, appearing in Table 1, concerns the first two intervals occurring between the first three broods discussed above. If a compensating process operates by-way-of shorter intervals between smaller broods, these data should reveal the effect. The nonrelated parents develop a lower mean than the first generation full-sib matings though significant differences are absent between all \( F \) values. The data indicate no time compensating effect for fecundity differences reflected by brood size.

Regression coefficients for day intervals on interval order number where successive

| Table 1. Fecundity statistics for four inbreeding coefficients. First line gives the mean or regression coefficient ± 1.96 (its standard error); \( n \) gives brood and interval numbers. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| \( F = 0 \)     | \( F = .125 \)  | \( F = .250 \)  | \( F = .375 \)  |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Mean brood size, first three broods | 9.54 ± 0.58 | 8.57 ± 1.06 | 7.46 ± 0.63 | 7.69 ± 1.06 |
| \( n = 168 \)   | \( n = 30 \)    | \( n = 93 \)    | \( n = 48 \)    |
| Regression coefficient, brood size, \( y \), on brood number, \( x \) | 1.388 ± 0.35 | 1.353 ± 1.13 | 0.847 ± 0.27 | 1.490 ± 0.71 |
| \( n = 237 \)   | \( n = 39 \)    | \( n = 177 \)   | \( n = 76 \)    |
| Mean days for first two brood intervals | 44.83 ± 3.33 | not applicable | 52.27 ± 7.64 | 41.83 ± 5.49 |
| \( n = 117 \)   | not applicable | \( n = 66 \)    | \( n = 36 \)    |
| Regression coefficient, day interval, \( y \), on interval number, \( x \) | –2.348 ± 2.08 | not applicable | –9.52 ± 3.53 | –2.169 ± 5.57 |
| \( n = 179 \)   | not applicable | \( n = 122 \)   | \( n = 47 \)    |
periods between broods 1 and 2 and broods 2 and 3 are intervals 1, 2, etc. appear in Table 1. All intervals have been considered in the regression analysis and all coefficients are negative; however, the only coefficient significantly differing from zero concerns the nonrelated parents at a $P < .05$. Failure of the full-sib group, $F = .250$, to generate a significant coefficient is noteworthy as the sample represents 122 intervals. Thus, day intervals between broods of nonrelated parents decrease and brood size for successive broods increases. First generation full-sib matings, an important comparison for monogeny, have no significant reduction of interval between broods and the increase in brood size is significantly less than occurs for nonrelated parents. Parents of half-sib matings suffer no significant loss of genetic representation in the subsequent generation though the data suggest a trend in that direction. Even though multiple mating occurs, full-sibs are still possible in each family and loss of fecundity results by their inbreeding.

The likelihood of frequent sib matings, a requirement for Howard's (1942) explanation, is low. General experience with the isopods shows that members of a brood are released at one site and mature over a period of 60 to 80 days, perhaps as low as 50 days for some males. During this maturation, they move about and have ample time to become randomly dispersed in the colony. The likelihood of an individual maturing sexually in the neighborhood of a full-sib is low though sib matings are more likely during the establishment of a new colony by a few founders. If these judgments on the prospects for sib matings are valid, then monogeny's maintenance must have another explanation.

**Monogeny and Selection**

R. A. Fisher's (1958, and earlier) explanation of sex ratios rests on the genetic representation of parents in subsequent generations. Their representation involves a relation between mating frequencies of the sexes, sex ratios of individual sibships relative to the ratio among breeding adults, and frequency-dependent selection. Each zygote is half male and half female in parental composition regardless of the adult sex ratio in the population. If mating frequencies are equal between the sexes, the sex of lowest frequency among adults contributes its genes in the highest value to the zygote population. Any genetic variation in sex ratio experiences selection favoring parents producing larger proportions of the sex currently at a low frequency among adults. The process predictably moves the zygote ratio toward a stable 1:1 value. Subsequent differential mortality giving unequal ratios may be balanced by more mating of the low-frequency sex whereby both sexes among adults are equally represented in the zygotes. Other processes may influence sex ratios but appear to play no part in isopod biology.

The principal assumption consists of an innate basis for variation in sex ratios of individual families. Studies cited above by Vandel, Howard, Juchault and Legrand clearly show this variation to be heritable for isopods. Fisher's view of sex ratios predicts some progenies in a system with monogeny may contribute their genes, and thus their parents' genes, in disproportionately low values to the succeeding generations. However, if monogeny does not lead to an unequal ratio for the full zygote population and if the probability of male and female survival is independent of sibship ratios, then a system with monogeny is similar to one where male and female probabilities are equal in each sibship. The suggestion is made below that selection will stabilize the zygote population ratio at 1:1 values even when monogenic females remain in the population.

Heterogeneity of unequal sex ratios with $F$ values was lacking in 87 sibships (with 2,621 total young) scored for sex. From this total, the number of males and females are as close as discrete units allow, 1,311 δ δ : 1,310 Ψ Ψ. The breakdown by $F$ value was:
Sex was scored at approximately 40 days of age though the high survival, 92–100 percent per brood, judged from the initial counts, testifies to the value's close estimate of the ratio at birth. Thus the isopod system with monogeny entails a 1 : 1 ratio for our closest estimate of the zygote value. For this property to exist, a balance must occur between arrhenogenic and thelygenic sibships. Since monogeny, on the grounds of dispersion and dispersal, plays a questionable advantage relative to inbreeding, one may wonder why it exists when the 1 : 1 zygote population can be maintained in a less complicated way by conventional amphogenic females. The infective, spirochete-like particule(s) appear to have seized upon isopod tissues as a host habitat and, to our knowledge, impose no other problem than unbalanced sex ratios. The particles have been documented in only a few species, but the similar properties of monogeny suggest the particle explanation is general. If total escape from the particle's invasion was impossible, isopods may have evolved a twofold response, arrhenogy and thelygeny. Thus a nuclear genetic switch may now exist with two routes and responding to selection where arrhenogeny and thelygeny stabilize at frequencies of .5. Vandel's data and my single observation cited in the Introduction support this prediction where 41 : 32, 4 : 4 and 5 : 4 numbers of arrhenogens to thelygenics respectively were found in females taken directly from nature. I suggest monogeny reflects a genetic polymorphism sensitive to the frequency-dependent selection operating on sex ratio variability.

The sex ratios in adult populations shift toward larger female proportions. Data exist for three populations, the compost and UF colonies in Gainesville and the colony in Manatee Springs State Park, Florida. The values are as follows:

<table>
<thead>
<tr>
<th>F value</th>
<th>Totals</th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>1,500</td>
<td>732</td>
<td>768</td>
</tr>
<tr>
<td>.125</td>
<td>34</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>.250</td>
<td>858</td>
<td>445</td>
<td>413</td>
</tr>
<tr>
<td>.375</td>
<td>229</td>
<td>115</td>
<td>114</td>
</tr>
</tbody>
</table>

2,621 1,310 1,311

Sex ratios for some isopod studies have reported 1 : 1 values among adults (Paris and Pitelka, 1962); however, Howard's (1942) survey is perhaps the most extensive. He sampled 19 populations of Armadillidium vulgare about Cambridge, England, finding only three to have less than 50 percent females and with a similar mean value to that found for V. evergladensis, 74 percent. The ratio for zygotes in A. vulgare is less easy to estimate. That species requires a long development period, and Howard appears to have scored his material at up to a year of age. Also, he did not make initial counts on brood size for these crosses and the extent of early mortality is unknown. His 1942 paper gives sex ratios, for another purpose, in Tables 3 and 4 for fifty-four families after duplicate broods in the two tables are removed. The total scored sample was 2,927 offspring having 1,674 females at .572 percent. This value significantly differs from
EVOLUTION OF ISOPOD SEX RATIOS

609

a 1 : 1 value (P < .001); however, it also differs from a female percent of .74 (P < .001). The longer interval prior to scoring perhaps allowed the higher female survival to be expressed in the laboratory stocks but these data suggest the early sex ratio is near 1 : 1. In these 54 families, a number of monogenic sibships existed, some of the extremes being 45 : 0, 26 : 0, 51 : 0, 0 : 49, 0 : 56, 0 : 53 male to female numbers respectively, yet they largely balance as with V. evergladensis.

SUMMARY

Monogenic females producing families significantly departing from 1 : 1 sex ratios exist among terrestrial isopods, in frequencies as high as 13.8 percent in Venezillo evergladensis. An infective, spirochete-like, cytoplasmic particle has been identified as responsible for the unequal ratios in sibships of individual females and is probably the general explanation for monogeny among isopods. The particle’s mode of action, while unknown, does not reduce fecundity by early mortality of specific sexes. Monogeny was earlier suggested as advantageous due to its potential for reducing inbreeding effects arising from full and half-sib matings.

A breeding study of V. evergladensis for a color polymorphism contributed data for brood size and interval between broods for four levels of inbreeding. First generation full-sib matings yield significantly lower fecundities than for nonrelated parents as measured by brood size. In addition, intervals between the smaller broods of the full-sibs do not compensate for their lower reproductive output per brood. Half-sib matings are not significantly lower in fecundity than for matings between nonrelated parents though the data suggest such a trend. The practice of multiple matings by females reduces the number of full-sibs per family, and the dispersal of young during maturation further reduces the likelihood of any sib mating. Another explanation for monogeny’s persistence seems necessary.

Sex ratio estimates are available for separate sibships where mortality following birth is judged insignificant. Individual monogenic sibships differ largely from 1 : 1 sex ratios but occur in numbers giving a 1 : 1 pooled sex ratio for the total zygote population. It is suggested that isopods have been unable to escape totally the spirochete particle invasion and have responded by evolution of a genetically controlled switch. The switch could control the direction taken by the sex ratio effect. Frequency-dependent selection predictably operates on females producing disproportionately larger numbers of male or female offspring where the two types stabilize at equal frequencies. Available data bearing on the hypothesis are reviewed.

LITERATURE CITED


TWO CONGRESSES

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