GENETIC SEGREGATION IN THE UNISEXUAL WASP DEVORGILLA¹

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THAT genetic mutations are prerequisite to evolution of the species is well recognized. The mechanism by which such mutations are segregated and recombined in bisexual species forms a large part of classical genetics. Less well known are the modifications, in unisexual species, of the same mechanisms to attain comparable ends. The segregation of genes in aberrant cases of diploid parthenogenesis has been described for *Habrobracon* (K. G. SPEICHER 1934), *Drosophila* (STALKER 1954; CARSON 1962), for the axolotl (LINDSLEY, FANKHAUSER, and HUMPHREY 1956) and for the honey bee (TUCKER 1958). In each of these cases normal reproduction is biparental. In this paper we analyze segregation in a species of wasp where males are virtually unknown.

Devorgilla canescens (Grav.), known also in the literature as *Nemeritis canescens* (Grav.), and more recently under the generic names *Idecthis* and *Exidecthis* (MUESEBECK, KROMBEIN and TOWNES 1951), is a cosmopolitan ichneumonid endoparasite of the Mediterranean flour moth, *Anagasta kühniella* (Zeller), and its relatives. Parasitization is accomplished by injection of an egg into the body of the host caterpillar, which remains active until pupation. Although many eggs may be laid individually in a single host (over 100 have been recovered), invariably only a single adult wasp emerges, a result of larval cannibalism.

Over a decade of breeding this species in our laboratory, and a considerably longer time spent by PROFESSOR S. E. FLANDERS at the Citrus Research Center, Riverside, California (FLANDERS and BADGLEY 1963), have failed to uncover a single functional male. One male was produced during the present experiment, from an X-rayed mother, but it was cytologically diploid and functionally sterile. Reproduction, then, is essentially, if not totally, thelytokous, at least in the strains studied. The authors are grateful to DR. FLANDERS for supplying the strain on which the present work was done.

Oogenesis in this species (described by B. R. SPEICHER 1937) should be understood for the purposes of this paper. Adult females have 22 chromosomes in diploid oogonial cells. The diploid number is retained in the nucleus of the matured, unfertilized egg by virtue of an abortive first maturation division. It is ineffective because the dyads, which would ordinarily remain at the periphery of the egg to form the first polar nucleus, return to the vicinity of the second

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oocyte nucleus to form a combined second metaphase plate with it. Cytological evidence is clear that the first maturation anaphase is reductional for the centromere, as indicated by the presence of separating dyads. The mode of segregation of the chromosomes in the second meiosis, whether reductional or equational, remains unclear from the cytological evidence.

When, in a single maturation division, the chromatids of a tetrad separate reductionally at the centromere, then in the absence of crossovers, all gene loci will separate reductionally as well, *AA* and *aa*. With one crossover between homologous strands, all gene loci distal to the chiasma will separate equationally. With two crossovers, the separation will be 50% equational and 50% reductional. MATHER (1935) derives the following general formula for determining the percentage of equational separations, E , after n crossovers: $E_n = \frac{2}{3}[1 - (-\frac{1}{2})^n]$. It may be noted that when $n = 0$ then $E = 0$, and when $n = \infty$ then $E = \frac{2}{3}$. Since the percentage of reductional separations, R , is $1 - E$, it follows that when $n = 0$ then $R = 100$, and when $n = \infty$ then $R = \frac{1}{3}$.

In *Devorgilla*, the situation is modified from MATHER's model by occurrence of the first, aborted, maturation division. This incomplete division has the effect of separating out dyads from the metaphase-I tetrads, then returning them to the common metaphase plate for second consideration in metaphase-II. With the first maturation division being reductional at the centromere, if the second division is equational, all heterozygous loci will remain heterozygous in the absence of crossing over.

On the other hand, if chromatid exchange does occur, then reductional separation for individual loci is possible, regardless of behavior at the centromeres, and the frequency of reductional separations will depend on the number of chiasmata formed between the centromere and the locus under study. Such chiasmata could not be determined cytologically, for the chromosomes of *Devorgilla* are too small for careful analysis. The problem, then, becomes a genetic one, and can be attacked by counting the offspring of heterozygous females. If segregation does occur, the proportion of homozygous offspring will indicate whether second division is equational or reductional. Also, the same proportion can be used to place the gene on the chromosome in its proper position in relation to the centromere. These considerations constitute the subject of the present paper.

MATERIALS AND METHODS

Mutations were induced by X-irradiating adult wild-type females at various dosages. The treated wasps were then put with host larvae, as their daughters were later, so that both daughters and granddaughters could be examined for visible mutations. One visible mutation (spread wings) was found among 1621 F_2 individuals, but this proved to be too variable in phenotype for genetic analysis. Eggs from 77 F_1 females were examined and three daughters of unrelated mothers which had been exposed to 600, 900, and 1000r, respectively, were found to be heterozygous for recessive lethal mutations that resulted in the production of inviable eggs. These were designated l_1 , l_2 , and l_3 and have been maintained in heterozygous stocks. Each shows individual characteristics as to time of action and frequency of appearance from a heterozygous female. However, only l_1 and l_3 were easily and unequivocally recognizable by the techniques employed.

These techniques consisted of permitting a heterozygous female to oviposit in a host cater-

pillar over a controlled period of time, then incubating the parasitized host for a period beyond the normal time for hatching of the eggs. The caterpillar was then either preserved in formalin or frozen and was later teased apart under a dissecting microscope to recover all of the inviable eggs and hatched larvae. Inviabile eggs were classified into stages according to the degree of development reached before death, hatched larvae were counted and recorded as viable.

RESULTS

Segregation of a simple recessive (l_1): This lethal behaves as a recessive point mutation. Its appearance in the homozygous condition indicates that the meiotic behavior in Devorgilla does permit segregation of heterozygous alleles. Listed in Table 1 are comparative counts and percentages of eggs that are inviable and viable (larvae) from the wild-type and l_1 stocks. Counts made from wild-type controls include all sibships that contain 20 or more eggs. On the other hand, counts from l_1 females were recorded only if they included 50 or more eggs in order to facilitate distinguishing, by progeny count, between the two phenotypically similar types of females that are expected to occur in the l_1 stock.

The proportion of eggs that die in stage 1 is essentially the same for both stocks. Stage 1 includes the majority of inviable eggs from the wild-type controls. They can easily be distinguished from stage 2 eggs, which are twice as great in diameter and have become rather opaque instead of translucent. Eggs that die in stage 1 are omitted from all subsequent discussion of l_1 since they occur as frequently in the wild type.

The majority of l_1 inviables die in stage 2, but they may die at later stages also, particularly at stage 4 immediately preceding hatching, probably a critical period. Stages 2, 3, and 4 are considered together and the difference between 9.59% (the proportion of eggs dead in these stages in l_1 stock) and 0.79% (in wild type) represents lethality caused by the l_1 mutation.

Table 2 includes egg counts from the 49 females of l_1 stock that produced 50 or more eggs (exclusive of stage 1) and were daughters of heterozygous mothers similarly well tested. The females are arranged in eight classes according to the percent of inviable eggs they produced.

Females heterozygous for l_1 will be expected to produce homozygous normal daughters $+/+$ as frequently as inviable eggs l_1/l_1 , and it is necessary to exclude the homozygotes in calculating percent inviability. No female of class 1 produced

TABLE 1

Production of inviable eggs in l_1 stock as compared with wild-type controls

	Number and frequency of eggs						
	Inviabile				Viable	Exclusive of stage 1	
	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Inviabilities	Totals
Wild-type stock	23 (2.22%)	4 (0.39%)	4 (0.39%)	0	1,006 (97.00%)	8 (0.79%)	1,014
l_1 stock	154 (2.15%)	503 (7.02%)	54 (0.75%)	115 (1.61%)	6,336 (88.47%)	672 (9.59%)	7,008

TABLE 2

Inviability of eggs from females whose mothers were heterozygous for l_1

	Classes by percent inviability	Females	Inviabile eggs	Total eggs	Percent inviability
1.	0-2.9%	6	2	523	0.4
2.	3-5.9%	2	5	110	4.5
3.	6-8.9%	8	46	576	8.0
4.	9-11.9%	12	92	876	10.5
5.	12-14.9%	8	83	615	13.5
6.	15-17.9%	8	89	551	16.2
7.	18-20.9%	3	57	297	19.2
8.	21-23.9%	2	34	155	21.9
Total (classes 2 to 8 only)		43	406	3,180	12.77

more than 2.0% inviable eggs and the average for class 1 was 0.4%, while no female of class 2 produced less than 3.77% inviable eggs and the average for class 2 was 4.5%. Therefore, females of class 1 were classified as homozygous normal.

The 43 females exclusive of class 1 are considered heterozygous. Among the eggs they laid, the proportion of inviables is 12.77%. If segregation of the heterozygous l_1 locus were completely random, 16.67% inviable eggs would be expected.

As previously stated, cytological evidence indicates that the first abortive meiosis is reductional at the centromere. Subsequently, an equational second division would result in no inviable eggs in the absence of crossing over, and crossovers would allow a proportion of inviables ranging from 0 to 16.67%. On the other hand, if the second division were reductional also (in effect completing the separation started in the abortive first division), then 50% of the eggs would be inviable in the absence of crossing over, and crossovers would reduce the proportion so that it would range from 50% down to 16.67%. This result is incompatible with the observed ratio of 12.77%. The fact that this ratio is less than, not greater than, 16.67% indicates that the reductional abortive first division is followed by a second division which is equational at the centromere.

The amount of homozygosis of the normal allele is represented in Table 2 by the six females of class 1. These constitute 12.24% of the females tested, which in turn represent 87.23% of a hypothetical population of which inviable eggs constitute 12.77%. Therefore, it can be calculated that homozygous females make up 10.71% of such a population. The number of such females is small and their calculated frequency is not statistically different from 12.77% ($\chi^2 = 0.17$, $df = 1$), therefore, the latter figure is used for subsequent calculations since it is more reliably based on a count of 3,180 eggs.

The proportion of inviable eggs multiplied by two then represents the observable crossover frequency between the l_1 locus and the centromere, and the value of 25.53 may be accepted as a minimum approximation of the genetic map distance between the two. Some double crossovers are likely to occur within this distance, making its actual length somewhat greater. RIZET and ENGELMANN (1949) devised the formula $E = \frac{2}{3}(1 - e^{-3D})$, based on the work of MATHER

TABLE 3

Production of inviable eggs by daughters of females heterozygous for l_3 , compared with wild-type controls

Females		Eggs			
Genotype	No.	Inviabile	Viable	Total	Percent inviable
$+/l_3$	21	290	449	739	39.24
$+/+$	1	0	60	60	0.0
$+/+$ (control)	110	23	639	662	3.48

(1935), for determining the distance from the centromere, if there is no interference and all possible crossovers occur, where E is the proportion of equational separation of a locus and D is its distance in Morgans from the centromere, which separates reductionally. Since in Devorgilla $\frac{1}{4}$ of the eggs from an equational separation are inviable, $0.1277 = \frac{1}{4} E$. Solving the equation gives a map distance of 48.44 units, which may be regarded as a maximum approximation since there is no way to estimate the amount of interference that occurs between chiasmata in this region. The actual map distance should then lie between 25.5 and 48.5 units.

Segregation of a chromosome aberration (l_3): Segregation data were obtained from heterozygous females by use of the same techniques employed for l_1 . The frequency of inviable eggs produced by normal wild-type controls (Table 3) was 3.47% of the total. This is in good agreement with the comparable control figure of 3.00% ($\chi^2 = 0.71$, $df = 1$) obtained for the l_1 experiment, in spite of the fact that the two sets of eggs were recovered by different investigators.

Eggs from 23 females, daughters of l_3 heterozygotes and laying 20 or more eggs, were counted. Each group of eggs was checked statistically against the wild-type controls for the presence of the l_3 character. One female among the 23 so tested could not be identified as to its genotype, and its 21 eggs were excluded from further calculations. One female alone, of the 22 remaining, was homozygous normal, $+/+$. She laid 60 viable and no inviable eggs. The 739 eggs laid by the remaining 21 heterozygous females showed an inviability frequency of 39.24%. Subtracting 3.47%, the inviability of wild-type stock, gives a net inviability of 35.77% due to the l_3 mutation alone.

The presence of inviable eggs proves segregation for the l_3 mutant, but l_3 obviously does not behave as a simple recessive gene like l_1 , from the following evidence. The frequency of inviable eggs exceeds the highest percentage (25%) expected on the basis of either reductional or equational separation of a locus. Also, if l_3 were a simple recessive gene, the percentage of homozygous dominants (genotypically normal daughters) should equal the percentage of homozygous recessives (inviable eggs). In actual fact, only one of the 22 tested females proved to be homozygous normal. After adding a figure of 14 expected inviable eggs to this total of 22 viable, one arrives at a calculated frequency of 2.78% homozygous normal. This makes an unlikely 1:1 ratio with the 35.77% inviable eggs.

These facts, coupled with the early time of death of l_3 eggs in stage 1, suggest

that L_s is caused by a radiation-induced chromosome aberration. If the aberration were in a balanced condition in the heterozygote, and crossovers produced various unbalanced genomes so that both crossover classes usually fail to survive, then the situation observed in L_s would follow. Several different types of aberration could provide these conditions. It is beyond the purpose of this paper to choose among them, and futile to try with the information at hand.

DISCUSSION

Most hymenoptera reproduce by arrhenotokous parthenogenesis, and this would seem to be the basic method in this order of insects. Activation of the egg to cleavage, often given as one function of the sperm, is here, in the absence of a sperm, of necessity accomplished by some other stimulus. Thus, the arrhenotokous egg is freed for evolutionary experiments in other types of parthenogenesis, and it is not surprising that independent cases of thelytokous parthenogenesis have arisen not infrequently throughout the group. The methods by which this is accomplished differ within the members of the order, both apomixis and various modifications of automixis being displayed. This variation in the cytological mechanisms employed to gain the same end emphasizes their independent origins.

Complete thelytoky must affect the operation of natural selection as compared with the ancestral arrhenotokous inheritance where undesirable genomes are continually selected against, especially in the haploid male. The cytological mechanism for meiosis in *Devorgilla* is such that genetic segregation must be slower than in arrhenotokous species for all recessive traits, but faster for most of them than in large panmictic populations of diploid, bisexual species. An exception to the latter would be those recessive genes which lie in proximity to the centromere. They would be segregated infrequently, or even not at all, and it is quite possible that *Devorgilla* females are more or less permanently heterozygous for a number of genes.

The tendency to remain heterozygous for proximal regions of the chromosome could be involved in sex determination, if *Devorgilla* follows the scheme proposed (WHITING 1943) for *Habrobracon*, where femaleness is determined by heterozygosity at a sex locus or region. In *Devorgilla* a pair of sex determining genes would only need to be virtually completely linked to their respective and homologous centromeres to ensure that all individuals would be heterozygous for the pair, and so potentially female. The one diploid male reported here, which occurred after X-irradiation of the mother, could be explained as due to a chromosome rearrangement that made the sex locus no longer heterozygous.

The accumulation in *Devorgilla* females of recessive lethal genes, closely linked to the centromere on homologous chromosomes, would practically ensure the elimination of haploid males should the thelytokous meiosis revert to the arrhenotokous plan. There would seem to be no return from thelytoky.

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SUMMARY

The ichneumonid *Devorgilla* reproduces by female parthenogenesis. Egg diploidy is retained by an abortive first maturation, where the separating anaphase dyads return to a common second metaphase. One polar nucleus is then eliminated, so that two chromatids of each original tetrad are retained in the egg and two are discarded. The segregation of X-ray-induced recessive lethals was studied by counting the inviable eggs from heterozygous females. One lethal behaved as a point mutation and produced 12.77% homozygous recessive (lethal) eggs. Homozygous normal females also appeared in an approximately equal proportion.—The frequency of homozygosis of each gene, being less than 16.67%, indicates that the centromeres involved separate reductionally at the first meiotic division and equationally at the second. The amount of detectable crossing over between the locus and the centromere is 25.53%. The actual map distance should be between 25.5 and a calculated 48.5 units. A second lethal mutation produced 35.77% inviable eggs and only 2.78% homozygous normal females. The disparity between these two classes, and the high total inviability, suggest the presence of a chromosome aberration.

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