REPRODUCTIVE ECONOMY IN THE CHALCIDOID WASP MELITTOBIA

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INTRODUCTION

MALE haploidy is characteristic of the insect order Hymenoptera, of the homopterous groups Aleurodidae and iceryine Coccidae, of the anomalous beetle Micromalthus, and possibly also of the Thysanoptera, of rotifers and of all Acarina except the suborder Mesostigmata. In a recent review of the literature (WHITING 1945) it was concluded that there had been shown no convincing evidence against the extension of the general principles of sex determination proved for Habrobracon to all other species with haploid males. However, in species naturally reproducing by close inbreeding, this would involve considerable wastage of eggs.

In Habrobracon, determination of the female sex is complementary with the female heterozygous for a pair of sex factors the members of which are separated in her sons of the two haploid genotypes. The homozygous combinations occurring in fifty percent of the fertilized eggs of inbred stocks result in sterile diploid males and excess bad eggs. In outcrosses this reproductive wastage is avoided because all fertilized eggs are female-producing. It was at first suggested that the reproductive economy of outcrosses might be brought about by differential maturation of the egg (WHITING 1935) or by multiple independently segregating factor pairs (SNELL 1935).

It was later shown (WHITING 1943) that neither nonrandomness in fertilization nor multiple factors is the true explanation for the high fecundity of outcrosses in Habrobracon, but that a series of multiple sex alleles (*xa*, *xb*, *xc*, etc.) exists so that in nature the majority of matings would involve three alleles ($xa/xb \times xc$). Natural selection would act against two-allele crosses ($xa/xb \times xa$) and favor the maintenance of several alleles in a population.

Habrobracon juglandis (Ashmead) is the only species in which multiple sex alleles have been proved, but genetic work on two other members of the genus, *Habrobracon brevicornis* Wes. (SPEICHER and SPEICHER 1940) and *Habrobracon pectinophorae* Watanabe (INABA 1939) and on the chalcidoid wasp *Pteromalus puparum* (DOZORCEVA reviewed by WHITING 1940) suggests that the same scheme may be extended to these insects.

However, in several species of invertebrates with haploid males there is normally a considerable amount of inbreeding. Because of this fact, multiple sex alleles or multiple sex factors, if they exist in such closecrossed species, would be inadequate to prevent reproductive wastage. In the cottony cushion scale *Icerya purchasi*, males are infrequent and the females have been secondarily modified into hermaphrodites with haploid sperm produced in the hermaphrodite ovary. Self-fertilization is the rule, although the hermaphrodite may mate with males so that outcrossing is possible (HUGHES-SCHRADER 1927, 1930).

DREYFUS and BREUER (1944) have studied the chromosomes of the proctotrypoid wasp *Telenomus fariai* (Lima), a parasite of the eggs of the "barbeiro" bug Triatoma. In this wasp close inbreeding is the rule, "daughters and sons of the same mother copulate before leaving the egg shell of the host. Despite the brother-sister mating, diploid males are unknown. This difference in the behavior of Habrobracon and Telenomus is in close relation with the remarkable cytological mechanism present in the latter form." According to the authors, females are heterozygous, XY, males are haploid, X, and differential egg maturation occurs retaining X in the egg. Regular loss of a chromosome fragment takes place in spermatogenesis changing X to Y so that all sperm carry Y.

Egg counts were made, viability ratios determined, and genetic studies with mutant traits carried out neither in *Icerya purchasi* nor in *Telenomus fariai*. The possibility therefore remains that inviable sex homozygotes might be produced although diploid males were not observed. It may be mentioned here that in several stocks of Habrobracon diploid males are of very low frequency due to their extreme inviability. It is doubtful whether diploid males occurring but one percent as frequently as females would be detected without a genetic marker, unless many specimens were examined cytologically. The high diploid male stocks of Habrobracon bred in the laboratory have been derived by selection. Such selection is regarded as reverse to what occurs in nature in the evolution of male haploidy in perfecting the haploid genotype. It is, moreover, uneconomical as regards food supply for the species, so that natural selection might act to reduce diploid males in two-allele crosses for this reason also. It may well be that in many haplodiploid species, all sex homozygotes are inviable.

MATERIAL AND METHODS

Four species of the chalcidoid genus Melittobia have been bred and studied considerably (references in SCHMIEDER 1933, 1938), and all are much alike in structural features, habits, and life history. The males are eyeless and short winged and also differ from the females in several other structural details and in color. They constitute but a small proportion of the population, usually not over three percent. The females parasitize the larvae of mud-dauber wasps stinging them and ovipositing upon them. Since the parasite is very small, not over two millimeters in length, hundreds of progeny may develop on a single host larva. Among these, the few males, often only one or two, mate with the large number of females, the majority of which thus become impregnated. Active oviposition occurs only if stimulated by mating. A virgin female deposits only a very few eggs which are invariably male-producing. Thus an unmated female finding a new host, may mate with her own son, and thereupon produce an abundance of daughters.

Failure of free oviposition without mating is evidently an adaptation for conservation of the food supply which would otherwise be exhausted in the production of superfluous males. Inbreeding is the rule, son to virgin mother or, more frequently, brother to sister. Outcrosses may, however, occur among the progeny if females from different sources by chance parasitize the same host. There would seem to be little or no possibility for the male to migrate from host to host, handicapped as he is structurally and by relatively short life span.

Because Melittobia belongs to a division of the Hymenoptera distantly related to Habrobracon and because of the difference in sex ratio, habits, and life history, it seemed of interest to determine whether or not and to what extent the principles demonstrated for Habrobracon are applicable for this genus. A series of experiments was therefore planned and carried out beginning in January 1945. The technical work, including both the maintenance of the stocks and the collection of data from the experiments, has been done entirely by SCHMIEDER.

Two different highly inbred stocks of a species as yet undescribed, Melittobia species-C, were used, one in the 87th inbred generation from material collected in Wabash, Indiana, the other in the 109th inbred generation of a stock from Marlton, N. J. In deriving these stocks, a single female was isolated every generation on one host and two or three sisters on another. If progeny were secured from the single female, which was usually the case, then the next generation was continued from these. Occasionally, however, it was necessary to fall back on the "mass culture" from the sisters, and thus it would be possible that the females of the next generation might mate, not with a brother, but with a cousin. Daughters of single females would mate necessarily only with brothers and frequently there would be but a single male in the brood. Theoretically then, except for the sex alleles, the females of each of the two stocks should be homozygous and the males should be similar to each other. All fraternities within a stock should be two-allele, but the outcrosses might be of the three-allele type. It might, therefore, be possible that average number of females (♀♀/eggs) and female ratio (♀♀/♂♂) from the outcrosses would be double those from the closecrosses if the two stocks differed in sex alleles.

In the experiments single females were transferred to new hosts every day or two, counts were made of eggs deposited, and records were taken of adult offspring developing. Percentages of eggs producing adults should be very little lower than hatchability percentages since post natal mortality is known to be very low.

NEW DATA

Table 1 gives the summaries according to types of matings made. The term selfcross will be used in this paper as a matter of convenience to designate the cross of a haploid son back to his mother. Genetically, it is presumably similar to self-fertilization in the hermaphrodite of *Icerya purchasi* with haploid sperm. It also resembles selfing in diploid species in that fifty percent of heterozygosis is thus eliminated.

The selfcrosses include progenies of five Wabash females after each was crossed to her own son and progenies of thirteen Marlton females each of which was similarly first set unmated and then crossed to her son. Two of the closecrosses excluding selfcrosses were within the Wabash stock and eight within the Marlton stock, all pairings being with brothers except one of the latter. The outcrosses included four of Wabash females by Marlton males and four of the reciprocal. The F_2 from the outcrosses were four from daughters of Marlton females by Wabash males, crossed with their brothers (Marlton) and four from

TABLE 1

Summaries according to types of matings.

	DAYS	EGGS	♀♀	♂♂	EGGS / DAYS	♀♀×100 / EGGS	♂♂×100 / ♀♀
Self crosses* (♀♀ 23–40)	131	7,611	6,976	200	58.1	91.7	2.9
Closecrosses excluding selfcrosses (♀♀ 1–6, 41–44)	83	5,044	3,405	94	60.8	67.5	2.8
Outcrosses (♀♀ 7–14)	67	4,034	1,980	50	60.2	49.1	2.5
F_2 from outcrosses (♀♀ 15–22)	79	4,765	3,088	81	60.3	64.8	2.6
Totals including later fraternities from females 1–19	454	25,559	18,671	512	56.4	72.9	2.7

* The term selfcross is used in this paper to designate mating of a haploid son back to his mother.

daughters of Wabash females by Marlton males, crossed with their brothers (Wabash).

With the totals of table 1 there were also included progenies produced by nine of the females which were mated to various males after they had exhausted their sperm supply from their first matings. About sixty eggs are laid per day by young females, but old females tend to lay fewer, as is suggested

TABLE 2

A selection from the detailed data showing successive broods and successive fraternities produced by some of the females. The three columns of figures represent numbers of eggs laid and of adult males and adult females developing in successive broods of different fraternities and in different fraternities produced by different females or by the same female mated to different males. A brood includes the progeny from eggs laid on a single host before the female was transferred to a new host. In the complete data the parent females are numbered from 1 to 44, the parental males from 1 to 59. W indicates a parent of inbred stock from Wabash, Indiana, M from Marlton, New Jersey, M/W ♀ a first generation hybrid female from Marlton mother by Wabash father, W/M ♀ from the reciprocal. ?♂ indicates *that the source of the male was not recorded. In the detailed data the number of progeny occasionally exceeds the number of eggs recorded. This is because a few eggs were missed as they were being counted.*

W♀ 1×brother 1

EGGS	♀♀	♂♂
57	22	1
77	15	0
156	20	0
137	5	0
128	109	2
161	74	3
168	90	2
106	62	2

W♀ 2×brother 3

EGGS	♀♀	♂♂
46	22	0
75	16	2
161	67	3

M♀ 3×brother 4

EGGS	♀♀	♂♂
42	23	1
78	3	0
170	8	0

M♀ 4×brother 5

EGGS	♀♀	♂♂
53	38	1
91	38	1
155	62	0
119	32	0
133	134	2
118	77	2
165	109	2
110	67	3

W♀ 7×M♂ 9

EGGS	♀♀	♂♂
56	12	0
104	34	0
132	46	1
106	27	1
124	92	3
96	92	2

W♀ 7×M♂ 10

EGGS	♀♀	♂♂
47	37	2
90	68	2

W♀ 7×M♂ 11

EGGS	♀♀	♂♂
86	64	5
137	125	3
43	18	2
70	29	3
82	49	1

W♀ 8×M♂ 12

EGGS	♀♀	♂♂
36	35	0
160	72	4
117	22	0
120	99	2
95	79	3

W♀ 8×M♂ 13 total 9 broods

EGGS	♀♀	♂♂
862	709	16

W♀ 9×M♂ 14

EGGS	♀♀	♂♂
5	2	0
92	53	0
164	91	2
132	24	0
124	93	2
65	51	4

W♀ 9×?♂ 15 total 4 broods

EGGS	♀♀	♂♂
433	396	10

W♀ 9×?♂ 16 total 3 broods

EGGS	♀♀	♂♂
237	192	7

W♀ 10×M♂ 17

EGGS	♀♀	♂♂
8	3	0
114	42	2
140	84	3
111	40	1
101	92	2
117	108	2
57	52	2
39	34	0
2	0	0

W♀ 10×M♂ 18 total 6 broods

EGGS	♀♀	♂♂
788	712	18

M♀ 11×W♂ 19

EGGS	♀♀	♂♂
16	4	0
110	8	0
129	59	1
121	38	0
112	91	2
6	3	2

M♀ 12×W♂ 20

EGGS	♀♀	♂♂
73	17	1
152	36	0
161	106	2
145	65	2
107	111	2
4	0	2

M♀ 12×M♂ 21 total 3 broods

EGGS	♀♀	♂♂
143	111	2

M♀ 12×M♂ 22

EGGS	♀♀	♂♂
146	77	2

M♀ 12×W♂ 23

EGGS	♀♀	♂♂
137	49	1
128	120	1
107	88	3

M♀ 12×son 24

EGGS	♀♀	♂♂
115	67	1
40	21	1
15	9	1
76	58	1
54	51	2
10	7	1

M♀ 13×W♂ 25

EGGS	♀♀	♂♂
40	5	2
124	1	0
141	36	0
115	13	1
31	25	4

M♀ 13×M♂ 26

EGGS	♀♀	♂♂
38	21	0
65	58	0

M/W♀ 15× brother 28

EGGS	♀♀	♂♂
101	50	1
106	90	3
153	135	4
141	51	0
152	144	6
2	0	0

M/W♀ 15×M♂ 29

EGGS	♀♀	♂♂
82	53	1

M/W♀ 16× brother 30

EGGS	♀♀	♂♂
58	46	1
75	67	2
115	107	2
104	59	2
119	111	2
105	85	2
52	9	0

M/W♀ 17× brother 31

EGGS	♀♀	♂♂
48	33	0
76	56	1
133	127	1

W/M♀ 19× brother 33 total 7 broods

EGGS	♀♀	♂♂
943	659	22

W/M♀ 19×?♂ 34

EGGS	♀♀	♂♂
19	14	0
67	4	0

W/M♀ 20× brother 35

EGGS	♀♀	♂♂
126	82	2
120	79	1
103	83	3
128	27	1
168	43	1
132	76	1
111	74	2
49	46	2
51	25	0
84	2	0
107	59	1
45	26	2

W/M♀ 21× brother 36

EGGS	♀♀	♂♂
173	110	2
134	96	3
134	123	2
138	69	1
200	72	3
99	56	2

in the selfcrosses and in the later crosses. Ratio of males to females proved to be somewhat less than three percent. It appears to fluctuate very little in the different groups of crosses shown in table 1 or even in the detailed data shown in table 2.

High fecundity of closecrosses

As regards eggs developing into females, however, there are wide differences. Data are inconsistent with expectation based on results from Habrobracon. Eggs from the closecrosses produce more females than those from the outcrosses instead of the reverse, and in the selfcrosses with more than ninety percent of the eggs producing adult females there is no possibility of an equal number of "bad" eggs which might correspond to the homozygous diploid male-producing combinations expected in two-allele crosses. F_2 progenies from the outcrosses closely approximate the offspring from the closecrosses. The data suggest that, on the average, the more closely related the parents, the higher the fertility of the eggs.

Fluctuating and uniform fraternities

The detailed data reveal some interesting conditions which cannot be brought out by the summaries. Hence table 2 has been prepared, representing a selection from these data and showing the different broods of some of the fraternities and also the different fraternities derived by different matings of some of the females. A brood is here defined as the progeny produced on a single host and thus developing from the eggs laid over a period of one or two days according as the mother was transferred from host to host. After a varying number of transfers a female would cease laying eggs, thus indicating exhaustion of her sperm supply. She might then be mated to a second male to produce a second fraternity.

The single fraternity produced by W ♀ 1 crossed with her brother consisted of eight different broods. Numbers of eggs laid for the first two broods were smaller than those from the later merely because transfers were made sooner. Offspring produced per egg fluctuate very widely, being especially low in the third and fourth broods but increasing suddenly in the fifth. After the eighth transfer only one egg was laid, indicating exhaustion of sperm supply.

The outcross of W ♀ 7 by M ♂ 9 resulted in six broods showing wide deviations in adults produced. After the female ceased ovipositing she was outcrossed a second and again a third time to Marlton males 10 and 11, respectively.

If there was very little deviation in viability among the broods of a fraternity, only the totals are given. Thus W ♀ 8, giving a fluctuating fraternity by M ♂ 12, gave a uniform fraternity by M ♂ 13 and W ♀ 9, after producing a fluctuating fraternity by M ♂ 14, gave two uniform fraternities by males of unrecorded origin. Some fraternities consist of but a single brood as the third produced by M ♀ 12. Her second was unform and her fifth, from a selfcross by son 24, was fluctuating, viability being about fifty percent in the first three broods but rising to near 100 percent in the fifth. The second brood from M ♀ 13 outcrossed to W♂ 25 resulted in but a single adult from 124 eggs, while the fifth gave 29 from 31 eggs. It is unnecessary to comment further on the selected data which may be studied in table 2.

Since broods from the selfcrosses of females 23–40 (table 1) were, in general, very uniform, showing insignificant deviation from the high average 91.6 percent, the detailed data are omitted from table 2. A selection of the more divergent broods showed four with females per egg about 71 percent (194 females from 271 eggs) and in one small brood there were only 29 females from 58 eggs.

The data suggest that the difference between fluctuating and uniform fraternities is genetic. The broods of the uniform fraternities are all of high viability, no fraternities being as yet obtained with broods of uniformly low viability.

The cause of the differences between broods within a fluctuating fraternity is unknown. An attempt was made to determine whether or not food might influence viability. Larvae of mud-dauber wasps may be collected in early spring and refrigerated until it is desired to use them. After several months they lose their capacity to metamorphose, but they serve well as Melittobia food for several years. Of ten Marlton females eclosing in March 1946, nine were mated with their own sons and one was mated with a Marlton male from another culture. Five had been fed before mating on *Trypoxylon politum* larvae collected in March 1945, and four had been fed on *Sceliphron caementarius* larvae collected in March 1942. After mating, some of the females were fed on Trypoxylon collected in 1943, 1945, and 1946 and Sceliphron collected in 1942 and 1946. The brood showing the lowest hatchability, 29 females and one male from 58 eggs, was 1942–Sceliphron fed, and one fraternity of eight broods totaling 686 females and 20 males from 744 eggs was 1946–Sceliphron fed. In general there was little association, however, between hatchability and type of host used but the experiment was carried out with crosses, mostly selfcrosses, giving relatively uniform fraternities of high viability.

As a further check on the possibility that condition of the host might affect hatchability, three sisters (M ♀ ♀ 42–44) were selected from one of the selfcross fraternities (M ♀ 32×son 47) and crossed with their brothers (M♂♂ 57–59). Females 42 and 43 were fed and their offspring reared on Sceliphron hosts collected in March 1943, the poorest, discolored, shrunken host larvae available being used. For female 44, relatively fresh *Trypoxylon clavatum* was used, mostly 1946 material. From the two "poorly fed" cultures there were produced 567 females and 16 males from 603 eggs and 201 females and five males from 215 eggs. From the well-fed culture there developed 504 females and 16 males from 538 eggs. Viability was uniformly high in the different broods of the three fraternities despite difference in feeding.

Offspring of unmated females

For the selfcrosses in the feeding experiment 11 Marlton stock females had been set unmated in March, 1946. The six that were fed *Trypoxylon politum* larvae collected in March 1945 laid 15 eggs, all of which developed into adult males. The five that were fed *Sceliphron caementarius* larvae collected in March 1942 laid 14 eggs, only six of which were viable. There is here a suggestion that viability of unfertilized eggs may be affected by age or by species of the host.

Only 29 eggs were produced in 120 days, the total active life of the 11 females before they were mated. This rate of 0.24 egg per day stands in marked contrast to the rate after mating, almost 60 per day.

Relative viability of males and females

Table 1 indicates no difference in sex ratio associated with the different types of matings, suggesting that viability of the males is positively correlated with that of the females. As a further check on this point the data from the fluctuating fraternities were analyzed according to broods. The broods were grouped according to percentage of eggs producing females. Seventeen broods of very low female production (1–20 percent) totalled 1,571 eggs; 21 of low female production (21–40 percent) totalled 2,174 eggs; 35 of intermediate (41–60 percent) totalled 3,616 eggs; 35 of high (61–80 percent) totalled 3,482 eggs; and 53 of very high (81–100 prcent) totalled 5,376 eggs. For the five groups the numbers of males were, respectively, 5, 15, 61, 66, 121, totalling 268. Percentages of eggs producing males were therefore, respectively, 0.3, 0.7, 1.7, 2.0, and 2.25. Production of males is positively correlated with production of females. A χ^2 test indicated no significant deviation in slope between the two distributions.

GENERAL DISCUSSION

Among closecrossed animals with haploid males, Melittobia is advantageous in that it is possible to count the eggs and subsequently make record of the progeny. In Telenomous and other egg parasites in which several individuals develop within a single host egg and mating occurs before emergence, it maybe argued that the Habrobracon scheme applies with wastage of 50 percent of the zygotes. Since it has now been established that this cannot be the case, in Melittobia at least, we may look for some adaptive mechanism associated with sex determination which has been developed for reproductive economy in other closecrossed species. Multiple allelism may be the more primitive and general method functioning for outcrossed species, with the closecrossed species adopting various modifications, but retaining female heterozygosis.

The data presented in this paper suggest that in Melittobia the closer the mating the higher the fecundity. If some type of non-randomness in fertilization has been developed for economy in inbreeding, it is possible that crossing of different stocks may so upset the balance that the mechanism fails to function perfectly, resulting in infertile eggs, homozygotes. The data are not yet adequate to establish that outcrosses are, in general, less fecund than closecrosses. Differences between the fluctuating and the uniform fraternities may be due to some untested environmental factor.

No diploid males have yet been found in Melittobia, all males studied cytologically having five chromosomes. Some of the infertile eggs produced with the fluctuating fraternities or even some of the few with the uniform fraternities may possibly represent sexhomozygotes.

The questions raised here may in time be answered if a sex linked mutant gene can be obtained.

SUMMARY

In an undescribed species of the chalcidoid genus Melittobia, close inbreeding is the rule, but outcrossing may occur. Unmated females lay very few eggs, and these are male producing. After mating occurs, an abundance of eggs is laid which, for the most part, develop into females, males constituting less than three percent of the offspring.

The experiments including egg counts show that despite close inbreeding with "selfcrosses" of haploid sons back to mothers, there are no diploid males and no "bad" eggs as in Habrobracon sex homozygotes. Moreover, outcrosses have been less fecund than closecrosses. The methods of reproductive economy and of sex determination have not been determined.

Fraternities fall into two groups as regards successive broods on different hosts—the uniform with broods of high fecundity and the fluctuating with some broods of very low fecundity. These differences appear not to be caused by food but may be genetic.

It is suggested that in haplodiploid species multiple sex allelism may be the more primitive and general method of reproductive economy and that the closecrossed species have adopted some other method.

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