BIPARENTAL MALES AND HATCHABILITY OF EGGS IN HABROBRACON[1]

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ARTHUR M. CLOUDMAN (WHITING and ANDERSON 1932, p. 423) made a comparative study of the fecundity of mated and unmated females of *Habrobracon juglandis* (Ashmead). The mean number of offspring from the vials producing males and females was 13.69 while the vials producing only males averaged 18.05. The difference, 4.39 ± 1.30,[2] is in favor of the unisexual fraternities. Thus some factor arising in connection with fertilization reduced the fecundity of mated females.

ANNA R. WHITING (WHITING and ANDERSON 1932, p. 425) made a series of observations on the fecundity of several kinds of females. Counts were made showing the number of eggs laid, the hatchability of the eggs, and the viability of the larvae. Females of various types produced approximately the same average number of eggs per day. Hatchability, larvae/eggs, and viability, adults/larvae, were each about 80 percent for females set as virgins or mated to unrelated males, while hatchability was greatly lowered (49.1 percent) and viability somewhat decreased when females were crossed to related males. This suggested that fertilization of eggs by related sperm renders some eggs incapable of developing very far. Since biparental males are known to be produced in crosses involving related stocks, where they can be distinguished from their azygotic brothers, the question as to a possible relation between the occurrence of biparental males and fecundity suggested itself.

The investigations reported at this time were undertaken in order (1) to determine the comparative fecundity of females bred as virgins or mated to males from one or the other of three different stocks; (2) to compare the hatchability of eggs and viability of later stages from virgins and females mated to the various kinds of males; (3) to determine what relation, if any, exists between fecundity and the occurrence of biparental males; and (4) to find any possible evidence to support the idea expressed by other investigators that biparental males are produced in a homozygous stock where they cannot be distinguished from azygotic males by their characteristics.

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[2] Standard errors are used throughout this paper.

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MATERIALS AND METHODS

Females used in all crosses belonged to stock 11-o, an orange-eyed stock derived by grading orange (from stock 3, Lancaster, Pennsylvania) up to wild-type stock 11 (Iowa City, Iowa). This stock was chosen because stock 11-o females crossed to stock 11 males produce a larger percentage of biparental males than any other stocks available. The females were divided into four groups: some were bred without being mated, some were mated to males of stock 11-o, some to males of stock 11, and others to unrelated males of wild-type stock 25 (New York City). Actual mating of the female and male was observed in each case, but two of the females mated to males of stock 11-o produced only male offspring. In the summary these females are grouped with those set without mating.

Females were set individually in small tin boxes, each lined with a piece of paper cut and fitted carefully, and containing a caterpillar which had already been paralyzed by another wasp. Each caterpillar was examined under a binocular to remove any eggs which might have been laid by the female which stung it. By using paralyzed caterpillars immobility was insured, giving the best conditions for egg laying and for subsequent examination. Observations were made daily on the contents of each tin box and the females were transferred to new boxes. Records were kept each day of the number of eggs laid by each female, the number of eggs that developed into larvae, and the number of larvae that reached the pupal stage. If it became apparent that one host was not going to be sufficient food for all the larvae on it, another paralyzed caterpillar was added to the box. When adults began to emerge, daily counts of the offspring were made. The mothers were transferred for an average of about thirteen days each. None was set after fifteen days because previous experience had shown that females usually exhaust their supply of sperm after fifteen or sixteen days and breed thereafter as virgins. Cultures were kept at 30° C except when observations were being made.

DATA AND DISCUSSION

In table 1 eggs/days represents egg production per female per day; larvae/eggs represents hatchability of eggs; pupae/larvae gives the percentage of pupation; and adults/pupae expresses the percentage of eclosion.

Virgin females. Summaries of data are given in table 1. All the offspring were males as expected.

Matings of unrelated stocks (stock 11-o females by stock 25 males). Hatch-

TABLE 1

STOCK 11-o FEMALES	NO. SET	TOTAL DAYS SET	EGGS	LARVAE	PUPAE	ADULTS ♂♂	ADULTS ♀♀	EGGS DAYS	LARVAE ×100 EGGS	PUPAE ×100 LARVAE	ADULTS ×100 PUPAE	BIPARENTALS ×100 TOTAL
Unmated	9	119	2501	1979	1921	1733		21.02	79.1 ± .81	97.1 ± .37	90.2 ± .68	
Mated to stock 25 males	8	109	2289	1905	1868	452	1306	21.00	83.2 ± .78	98.0 ± .32	92.5 ± .61	74.3 ± 1.05
Mated to stock 11 males	6	80	1682	1179	1086	393 140*	436	21.03	70.1 ± 1.1	92.1 ± .78	89.2 ± .94	59.4 ± 1.6
Mated to stock 11-o males	7	99	2022	1351	1245	560	523	20.42	66.8 ± 1.05	92.2 ± .73	87.0 ± .95	48.3 ± 1.5

* Biparental males, 24.3 ± 1.8 percent of total biparentals.

ability of the eggs and percentage of pupation were about the same as for virgin females. Of the offspring, orange-eyed males and black-eyed females, 74.3 ± 1.05 percent were females, indicating that the majority of the eggs were fertilized.

Matings of related stocks (stock 11-o females by stock 11 males). The offspring were orange-eyed males (azygotic), black-eyed males (biparental), and black-eyed females. Of the offspring 59.4 ± 1.58 percent were biparentals, and 24.3 ± 1.79 percent of the biparentals were males. A significantly lower proportion of the offspring were biparentals than from the cross to males of stock 25. Hatchability was also significantly less.

Matings within one stock (11-o females by 11-o males). The offspring consisted of orange-eyed males and females. Since the parents were similar in eye-color, biparental males, if present among the offspring, could not be distinguished by inspection from their azygotic brothers. The females laid practically the same number of eggs per day as the females in each of the other experiments. Hatchability was somewhat lower and there were fewer distinguishable biparentals than from other matings.

Presence and type of sperm failed to affect the egg productivity since females of all four groups laid approximately the same number of eggs per day. Any differences among the groups are due then to fertilization. Virgin females and those crossed to unrelated males of stock 25 give similar results, in that about the same percentage of eggs developed into larvae. From females mated to related males of stock 11 there is a much lower hatchability of eggs. Among the adults from this cross were biparental

males. It is very interesting that associated with lowered hatchability of eggs and lessened viability of later stages there is the production of biparental males, while associated with higher hatchability and viability there is an absence of biparental males. Thus it seems that whatever mechanism causes the production of biparental males simultaneously renders a considerable portion of the eggs and later stages incapable of further development. This suggests that the production of biparental males may be either due to or followed by some abnormal arrangement of the chromosomes, which prevents the development of certain individuals, and strongly supports WHITING'S (1933) explanation of the mode of sex determination and the occurrence of biparental males.

The crosses within the stock (to 11-o males) gave results strikingly similar to those from the cross to related males of stock 11. In table 1 it may be seen that for egg hatchability the two crosses are similar, in that the values for each are significantly different from those from the offspring of virgin females or females mated to unrelated stock 25. The percentages of eggs becoming adults are for the stock 11-o and stock 11 crosses 53.6 ± 1.1 and 57.6 ± 1.2, respectively, while for the outcross the value is $76.8 \pm .88$. Further comparison shows for the stock 11-o and stock 11 crosses biparental ratios of 48.3 ± 1.52 and 59.4 ± 1.58, respectively. The corresponding value for the outcross is 74.3 ± 1.05. All these values are significantly different.

Comparison of these figures leads one to believe that although biparental males cannot be detected, they are being produced, because the low hatchability of eggs and poor viability of later stages are very similar to that shown by the cross where biparental males are detectable. Since egg hatchability is 3.3 ± 1.5 percent lower than in the cross to stock 11 males, it suggests that even more biparental males are being produced. The lower ratio of distinguishable biparentals in this cross is due then to the inclusion of biparental males with the azygotic males.

Further evidence that biparental males are present among the offspring of the females crossed to like males was obtained by making fertility tests. It has been shown by several investigators that a large proportion of biparental males is sterile, and that even those which show some fertility cause the production of very few daughters. Seventy of the male offspring from stock 11-o by stock 11-o were selected at random and mated to virgin females. All the matings were carefully observed. Eighteen of the females produced only male offspring, which indicated that the males which were mated to these females were sterile. The smallest number of males produced by any female was 12, while the average was 30. Seven matings produced male and female offspring as follows, males being given first: 19, 2; 27, 1; 22, 3; 35, 1; 29, 2; 15, 1; 30, 2. The males used in these matings

were probably biparental also, as judged by the small number of daughters produced. Forty-five of the matings produced males and females in large numbers, the mean number being 6.7 males and 26 females. There is ample justification for the conclusion that the sterility of the majority of the twenty-five males, or about 36 percent of those tested, was due to their biparental constitution.

It should be noted that about the same proportion of larvae became pupae among the offspring of the virgin females and those mated to unrelated males of stock 25. On the other hand, significantly fewer larvae became pupae in the crosses to related stocks 11 and 11-o. Excess pupation among offspring from virgin females over offspring of crosses to stocks 11 and 11-o was $4.97 \pm .87$ percent and $4.92 \pm .83$ percent, respectively. Excess pupation of the outcross was $5.87 \pm .84$ and $5.82 \pm .80$, respectively. Similarly fewer pupae became adults in the crosses to stocks 11 and 11-o.

Eggs and pupae were less viable from the cross to stock 11-o males, showing that a greater proportion of individuals is rendered incapable of development than in any other cross. In the light of this and other evidence, the conclusion is fully justified that biparental males were produced in this cross. This conclusion also suggests that other inbred stocks in Habrobracon produce biparental males which are not recognizable.

DZIERZON'S LAW

DZIERZON many years ago advanced the theory that all the eggs produced in the ovary of queen bees are alike; those developing without fertilization become males, and those fertilized become females. Because not all the eggs of an unfertilized queen hatch, some have believed DZIERZON'S theory to be partly incorrect. One sentence will be quoted from PHILLIPS' text of Beekeeping (1922): "It seems clear, however, that the statement of DZIERZON that all the eggs in the ovary are male eggs cannot be accepted and it is, in fact, not improbable that the eggs destined to be females die for want of fertilization, while the eggs destined to be males, not requiring fertilization, are capable of development." Somewhat the same idea is expressed in the revised edition of PHILLIPS' text (1928): "The author has found that many eggs laid by drone-laying queens fail to hatch and, in fact, are often removed in a short time by the workers. This makes it impossible to accept DZIERZON'S statement that all eggs laid by such a queen become males and the statement must be modified as follows: all of those eggs laid by a drone-laying queen which develop become males. The potentialities of the eggs which never hatch are not known."

For the first time, as far as is known to the author, actual egg counts for mated and unmated females have been made for an insect probably com-

parable to the honey bee. There can be no doubt that in Habrobracon all the eggs of a virgin are alike for sex. Nine unmated females laid 2,501 eggs, of which 79.1 ± .81 percent hatched into larvae. Of the eggs 69.3 ± .92 percent were able to develop into adults, all males. Eight females, mated to unrelated males, laid 2289 eggs, of which 83.2 ± .78 percent became larvae and 76.8 ± .88 adults. Of the adults, 74.3 ± 1.05 percent were females. If the 21.9 percent of the eggs from unmated females failed to hatch because they were destined to be females, an unusual mechanism for the segregation of eggs for sex has occurred to give such a ratio. In addition, if the 21.9 percent failed to hatch because they were destined to be females, why should 57.1 percent of the eggs from mated females hatch and develop into females? Recent investigations have served to corroborate the views of DZIERZON, adding to his beliefs the knowledge that occasionally males are biparental.

SUMMARY AND CONCLUSIONS

1. Females of stock 11-o lay the same number of eggs whether bred as virgins or mated to males of the same stock, of a related stock, or of a totally unrelated stock.

2. Hatchability of eggs is high from virgins and from matings involving unrelated stocks. Hatchability of eggs is lower from crosses of related stocks, in which biparental males are known to be produced and within a stock in which the evidence indicates they are produced.

3. The percentages of hatchability of eggs, pupation of larvae, and eclosion of pupae are very nearly the same in the crosses to males of related stocks 11 and 11-o, although lower in the latter. Thus it seems likely that biparental males are being produced in the latter cross, where they cannot be distinguished in appearance from their uniparental brothers, as well as in the former cross, where they are distinguishable.

4. Further evidence for the production of biparental males by inbreeding of stock 11-o was shown by the sterility of about thirty-six percent of the male offspring tested.

5. Although the causes underlying the production of biparental males are not known, it may be of considerable value to know that associated with the appearance of such males is a decreased hatchability of eggs and viability of offspring.

6. All the eggs produced by a virgin female are equivalent for sex.

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