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DURATION OF PAIRING AND USE OF ALLOSPERM IN *BIOMPHALARIA GLABRATA* (GASTROPODA: PLANORBIDAE)

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Virgin homozygous black pigmented and albino Biomphalaria glabrata *are paired during a period varying from 1 to 20 days. The rate of cross-fertilized parents is statistically similar for the various lengths of pairing. As a whole, nearly 80% of the albino snails produce a pigmented progeny. This production begins as soon as the snails are mated and continues after their separation. To measure the actual use of the allosperm, its use during the postmating period must be added to the length of mating. So, it appears that the real use of the allosperm is statistically constant (mean slightly inferior to 8 weeks) and not related to the length of the previous pairing.*

Key words: *Biomphalaria* – pigmentation – albinism – selfing – crossing – allosperm

Mature *Biomphalaria glabrata* show a simultaneous hermaphroditism. If they are reared in isolation, snails reproduce by selfing. When they are mated, snails copulate and reproduce by crossing (Brumpt, 1941; Paraense, 1955).

When snails are mated, the percentage of cross-fertilization does not reach 100%. Even after a short duration of mating (1 day), the crossed snails use allosperm during a long time which sometimes oversteps three months (Paraense, 1955, 1956; Vianey-Liaud et al., 1987).

In the laboratory, couples of *B. glabrata* may be mated for a varying length of time. The aim of this paper is to investigate if different durations of mating affect the rate of cross-fertilized snails and the length of the use of allosperm.

MATERIALS AND METHODS

The snails used were brazilian *B. glabrata*. Snails of two genotypes were bred separately from hatching:

– black pigmented snails: homozygous CC;
– albino snails: homozygous cc (Richards, 1967).

After having reached sexual maturity and reproduced by selfing, black pigmented and albino snails have been paired for a period varying from 1 to 20 days. For each duration of pairing (1, 2, 4, 6, 8, 10, 15 and 20 days), 10 couples have been constituted. The snails used showed a shell diameter varying from 13 to 15 mm; the difference of size between two paired snails never exceeded 1 mm.

After pairing, every albino snail was isolated and its progeny regularly controlled until exhaustion of the allosperm. The presence or the lack of pigmentation in the embryos' eyes indicated that the albino parent had reproduced by crossing or by selfing.

On the whole, 80 pigmented and 80 albino snails were mated. Snails were raised in isolation (except during mating) in 65 ml of tap water and fed with fresh lettuce. They were maintained at 25 °C, in artificial light (12L/12D). During the experiment, 3,618 egg clutches were monitored.

RESULTS

After mating, the offspring of the albino snails is regularly controlled. In these experiments, only cross-fertilizations due to the albino snails acting as a female are taken into account. For each duration of mating, the albino parents produce pigmented progeny, albino progeny or are sterile or dead (Table I).

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TABLE I

Analysis of the offspring of albino *Biomphalaria glabrata* mated with black pigmented snails during various lengths of time. For each duration, 10 couples have been constitued

Duration of mating (days)	1	2	4	6	8	10	15	20	Sum
No. of albino producing pigmented progeny	8	6	8	8	8	7	8	9	62
No. of albino producing albino progeny	0	1	0	0	0	0	1	1	3
No. of albino laying no clutch or dead	2	3	2	2	2	3	1	0	15

The results can be tested by the chi-square test. The value of chi^2 (9.44 for 14 degrees of freedom) does not reach the level of probability $p = 0.05$. For the various lengths of pairing, the rates of the three types of albino parents are to be regarded as not significant. It must be emphasized that the percentages of albino snails producing pigmented or albino offspring are not statistically correlated to the durations of matings ($r = 0.55$ and $r = 0.51$ for 6 degrees of freedom).

On the whole, 62 albino parents (= 77.5%) produce pigmented progeny until the exhaustion of the allosperm (Table I). After separation of the snails, the maximum usage of allosperm is monitored for each duration of mating (Table II).

After a logarithmic transformation of the data, the analysis of variance shows that the means are not statistically different: $F = 1.27$ for 7 and 54 degrees of freedom ($F\ 0.05 = 2.18$). The durations of the use of the allosperm show a significant linear correlation with the durations of matings ($r = -0.29$ for 60 degrees of freedom). The regression can be tested by an analysis of variance upon the regression (Table III).

TABLE II

Use of allosperm by albino *Biomphalaria glabrata:* – after separation of couples previously paired for various periods; – as soon as snails are paired

Length of pairing (days)	n	Postmating period			Mating + postmating period
		Use of allosperm (days)	Extreme data	Number of clutches	Actual use of allosperm (days)
1	8	54.3 ± 8.4	(12-85)	44.3 ± 5.6	55.3 ± 8.4
2	6	58.8 ± 11.7	(26-89)	45.8 ± 11.0	60.8 ± 11.7
4	8	51.0 ± 12.2	(08-94)	47.3 ± 13.6	55.0 ± 12.2
6	8	49.4 ± 8.9	(16-91)	39.8 ± 7.7	55.4 ± 8.9
8	8	46.9 ± 8.7	(12-89)	37.9 ± 7.3	54.9 ± 8.7
10	7	32.3 ± 11.6	(04-85)	27.6 ± 9.5	42.3 ± 11.6
15	8	40.0 ± 4.8	(16-54)	32.6 ± 4.7	55.0 ± 4.8
20	9	34.7 ± 6.5	(12-72)	30.4 ± 5.9	54.7 ± 6.5

n = number of crossed albino snails.

TABLE III

Analysis of variance on the maximum length of the use of the allosperm transferred after crossing to albino *Biomphalaria glabrata*

	S. S.	D. f.	M. S.	F	
Total variation	39546	61	–	–	
Linear terms	3351	1	3351	5.18	<0.05
Non linear terms	1225	6	204	0.31	n. s.
Error	34970	54	647	–	

D. f.: degrees of freedom; M. S.: mean square; S. S.: sum of squares; <0.05: linear terms of the regression significant to the level of probability P 0.05; n. s.: non linear terms of the regression no significant.

The linear terms of the regression are significant while the non linear terms are not significant. It appears that the maximum duration of the use of the allosperm (after separation of previously paired snails) decreases statistically linearly when the matings increase from 1 to 20 days. The slope of the regression line is negative: $b = -1.167$ and the standard deviation of the slope is 0.513. Consequently, there is a confidence of 95% that the decrease of the maximum use of the allosperm after mating decreases between –0.13 and –2.19 days for a daily increase of the length of mating.

How many egg-masses are laid during the use of the exogenous sperm? The results are presented in the Table II. After separation of the snails, the numbers of clutches laid until exhaustion of the allosperm are statistically similar in the various groups: $F = 0.75$ ($F\ 0.05 = 2.18$). These numbers are constant and not affected by the length of mating ($r = -0.14$ for 6 degrees of freedom). On average, 38.0 ± 2.9 egg-masses are necessary for a total depletion of the exogenous sperm.

During mating periods, snails deposite egg clutches. Except in the case of non-crossed albino snails (3.75%), all the clutches containing viable embryos exhibit a pigmented progeny. These embryos are produced by pigmented parents after crossing or selfing, but also by albino parents after crossing, suggesting that they are produced as soon as copulations occur. Therefore, the duration of the pairing period must be added to the postisolation period to evaluate the actual duration of the use of the allosperm (Table II).

In comparison with the data previously obtained after isolation of the snails, each mean is increased of the length of the mating, the standard error remaining similar. The statistical analysis gives opposite results. The linear correlation coefficient is not significant ($r = 0.04$ for 60 degrees of freedom). The regression coefficient is $b = -0.167$ and the standard deviation of the slope is 0.513. So, the fiducial limits of the regression coefficient, for the probability $p = 0.05$, are positive and negative ($-1.020 < b < +0.869$). The 8 means do not differ statistically: $F = 0.71$ ($F\ 0.05 = 2.18$). The general mean is: $m = 54.2 \pm 3.1$ days ($n = 62$).

It can be concluded that the real usage of the allosperm by albino snails fertilized by pigmented partner, remains constant and is not affected by the length of mating.

DISCUSSION

Even in large groups of snails, as a general rule, one *B. glabrata* copulates at a given moment with only one partner (*). When two snails are experimentally paired, copulation is unilateral, each snail acting either as a male or as a female.

The genetical exchanges between snails may be displayed by using albinism as a genetic marker (Newton, 1954, 1955; Paraense, 1955, 1956; Richards, 1970; Vianey-Liaud et al., 1987). If a pigmented snail copulates as a male, crossing may be easily detected by examining the pigmentation of the offspring produced by the albino partner.

In such experimental conditions, crossing does not affect all the paired couples (Table I). Only 62 albino snails (77.5%) produce a pigmented progeny; this percentage is similar to that observed in other experiments (Vianey-Liaud et al., 1987). If the usage of the allosperm is measured after separation of the snails, it appears that the mean durations statistically decrease when the lengths of the matings increase (Tables II & III). The use of the

(*) In a small number of cases, if reared with several partners, a snail may copulate simultaneously as male and female with two partners acting respectively as female and male (personal observations) (Brumpt, 1941).

allosperm received from the donor partner shows a great variability (Table II). This duration varies from 4 to 94 days, the larger data being slightly inferior to that observed previously (Vianey-Liaud et al., 1987).

The albino snails cross-fertilized by a pigmented partner produce pigmented heterozygous embryos immediately after being mated. So, use of the allosperm begins as early as the snails are paired and its duration must include the length of the mating period. Whatever the mating period may be, so defined, the total use of the exogenous sperm appears to be statistically constant. In the various groups, the maximum duration is very similar (Table II). The general mean is consistent with the previously observed values (Paraense, 1955; Vianey-Liaud et al., 1987).

In *Lymnaea stagnalis,* Van Duivenboden et al. (1985) and Van Duivenboden & Ter Maat (1985) have studied the copulatory activity. They show that a snail acting as a male acts as a female in the second copulation while snails acting formerly as a female do not show preference for any role. When a snail copulates successively as female, the oviposition rate decreases.

In our experiments, when two snails are paired, it appears that nearly from 80% of albino snails are cross-fertilized. This percentage is observed as soon as on the first day and remains statistically constant all along the experiment. This proves that snails copulate as soon as they are paired. Statistically, after one copulation 50% of albino snails may be cross-fertilized. The percentage observed is always higher but never reaches 100%.

Several hypotheses could be conceived. The duration of use of the allosperm may be proportional to the quantity of sperm transferred, therefore to the numbers of copulations. On this assumption, the use of the exogenous sperm would be proportional to the length of mating. The present experiments show obviously opposite results. The real use of the allosperm remains statistically constant when the duration of the matings increase. It appears that, statistically, whatever the mating period may be, the crossed snails deplete the allosperm after a similar duration. This may indicate that the quantity of sperm transferred does not depend on the number of copulations. It is difficult to imagine that the quantity of sperm transferred during every copulation is reduced if the number of copulations increases. As a matter of fact, when a snail is cross-fertilized, it does not know if copulations will subsequently occur. The number of copulations probably remains constant when the length of the mating period varies.

According to the results of Van Duivenboden & Ter Maat (1985), if several copulations occur, each snail theoretically plays successively the two roles. After two or more copulations, all the albino snails would have been cross-fertilized and produced pigmented progeny. This never occurs even if the length of the mating is multiplied by 20. The results obtained in *Lymnaea stagnalis* cannot be applied without alterations to *B. glabrata.*

Previous observations show that, in *B. glabrata,* the copulation occurs as soon as non copulant mature snails are paired. It is possible that, contrarily to all expectations. a copulation prevents or restricts further crossings. It will be necessary to confirm this hypothesis by studying the copulatory behavior of snails of two strains mated (here albino and black pigmented strains) and verifying if they preferentially act as male or female.

RESUMO

Duração do acasalamento e uso do aloesperma em *Biomphalaria glabrata* (Gastropoda: Planorbidae) — Espécimes virgens de *Biomphalaria glabrata* (um melânico e um albino) são acasalados durante um período variável de 1 a 20 dias. A proporção de espécimes parentais albinos fecundados por cruzamento é semelhante estatisticamente para os diversos períodos de acasalamento. Em conjunto, cerca de 80% dos albinos produziram descendência pigmentada. Essa produção começa assim que os moluscos são acasalados e continua depois que são separados. Para medir a utilização real do aloesperma, sua utilização durante o período de pós-acasalamento deve ser somada à duração do acasalamento. Parece, assim, que a utilização real do aloesperma é estatisticamente constante (média ligeiramente inferior a oito semanas) e não relacionada à duração do acasalamento anterior.

Palavras-chave: *Biomphalaria* – pigmentação – albinismo – autofecundação – fecundação cruzada – aloesperma

REFERENCES

BRUMPT, E., 1941. Observations biologiques diverses concernant *Planorbis (Australorbis) glabratus* hôte intermédiaire de *Schistosoma mansoni. Ann. Parasitol. hum. comp., Fr., 18:* 9-45.

DUIVENBODEN, Y. van; PIENEMAN, A. & TER MAAT, A., 1985. Multiple mating suppresses fecundity in the hermaphrodite freshwater snail *Lymnaea stagnalis:* a laboratory study. *Anim. Behav., G.-B., 33:* 1184-1191.

DUIVENBODEN, Y. van & TER MAAT, A., 1985. Masculinity and receptivity in the hermaphrodite pond snail, *Lymnaea stagnalis. Anim. Behav., G.-B., 33:* 885-891.

NEWTON, W., 1954. Albinism in *Australorbis glabratus. Proc. helminthol. Soc. Washington. 21:* 72-74.

NEWTON, W., 1955. The establishment of a strain of *Australorbis glabratus* which combines albinism and high susceptibility to infection with *Schistosoma mansoni. J. Parasitol., USA., 41:* 526-528.

PARAENSE, W. L., 1955. Self and cross-fertilization in *Australorbis glabratus. Mem. Inst. Oswaldo Cruz, 53:* 285-291.

PARAENSE, W. L., 1956. A genetic approach to the systematics of planorbid molluscs. *Evolution, USA, 10:* 403-407.

RICHARDS, C. S., 1967. Genetic studies on *Biomphalaria glabrata* (Basommatophora: Planorbidae), a third pigmentation allele. *Malacologia, USA., 5:* 335-340.

RICHARDS, C. S., 1970. Genetics of a molluscan vector of schistosomiasis. *Nature, G.-B., 227:* 806-810.

VIANEY-LIAUD, M.; DUPOUY, J.; LANCASTRE, F. & NASSI, H., 1987. Genetical exchanges between one *Biomphalaria glabrata* (Gastropoda: Planorbidae) and a varying number of partners. *Mem. Inst. Oswaldo Cruz, 82:* 457-460.