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Obligatory parthenogenesis in the stick insect Bacillus rossius (Rossi)

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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Zoologia. — *Obligatory parthenogenesis in the stick insect* Bacillus rossius (*Rossi*) (*). Nota di Valerio Scali, presentata (**) dal Corrisp. M. Benazzi.

RIASSUNTO. — Il fasmide *B. rossius* ha un areale di distribuzione circummediterraneo e si riproduce partenogeneticamente a nord (Francia meridionale e Italia nord-occidentale), mentre è anfigonico a sud (nord Africa e Italia meridionale). Non ci sono differenze citologiche e non sembra esistere alcuna barriera riproduttiva fra le popolazioni francesi e nord africane cosicché in ambedue i tipi di popolazione (uni- e bisessuata) le femmine possono venir fecondate e dare una discendenza con normale sex-ratio, oppure, se isolate dai maschi, riprodursi partenogeneticamente.

Tuttavia nell'Italia centrale, dove esistono ambedue i tipi di popolazione, è stato possibile isolare femmine che, pur ripetutamente accoppiate con maschi diversi, hanno prodotto una prole esclusivamente femminile o quasi, comportandosi così come « partenogenetiche obbligatorie ». Indagini citologiche sullo sviluppo embrionale precoce delle loro uova hanno rivelato che la segmentazione inizia col numero aploide di cromosomi come è la regola per le uova partenogenetiche. L'allevamento per cinque generazioni di un ceppo derivato da una di tali femmine « partenogenetiche obbligatorie » (ceppo 671) ha mostrato che la caratteristica di produrre solo femmine anche dopo numerose copule è costante e comune a tutte le femmine investigate. Ricerche citologiche collaterali suggeriscono che le uova di tali femmine non sono fecondate per la mancata penetrazione dello spermio. Infatti, mentre è stato possibile trovare una o più teste spermatiche (fino ad un massimo di 7) nelle uova di femmine anfigoniche, non è stato possibile ritrovare teste spermatiche in 72 uova del ceppo 671 esaminate con lo stesso metodo.

Il reperto di femmine con partenogenesi obbligatoria permette alcune reinterpretazioni di precedenti esperimenti e suggerisce nuove considerazioni per la comprensione della distribuzione delle popolazioni unisessuate e bisessuate in natura.

INTRODUCTION

The Mediterranean stick insect *Bacillus rossius* is parthenogenetic in the northern part of its range and amphigonic in the southern one (Tunisia, Algeria, Morocco). More detailed investigations on its reproductive biology were started first in France (Cappe de Baillon, Favrelle and Vichet, 1937; 1938) where populations consist only of thelytokous females; for this reason the species was thought to reproduce by obligatory parthenogenesis. At the same time however, Favrelle and Vichet (1937) crossed a North African male with thelytokous French females which were fertilized and so proved

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(**) Nella seduta del 14 novembre 1970.

to be facultatively parthenogenetic. Actually this result was a logical outcome of the normal meiosis of the egg already described by von Baehr (1907, 1920) in parthenogenetic females.

In Italy up to 1960 only an amphigonic population from Campania was known (see Montalenti and Fratini, 1959) and some reports about a possible parthenogenetic population from Liguria (North-Western Italy) were available.

Benazzi and Scali (1964) noticed that on a strip of land along the sea near Pisa, both males and females could be found. On the basis of collecting and breeding results for 1962 and 1963 it was possible to state amphigony and parthenogenesis coexisted in nature. Extensive collections by Bullini (1966, 1968, 1969) have shown that in Liguria and Tuscany both amphigonic and parthenogenetic populations can be found, the two kinds of populations being often quite close to each other and mixed without a clear distribution pattern over a large area along the coast. Some females, among those collected in 1963 at Marina di Pisa behaved in a peculiar way: although they normally copulated with males they had a progeny with daughters far more numerous than sons; for instance couple n. 1 gave 3 ♂♂ : 227 ♀♀ (Scali, 1968). Three daughters of this offspring, taken at random, were mated with several males: their progenies were respectively: A, 2 ♂♂ : 120 ♀♀; B, 2 ♂♂ : 100 ♀♀; C, 0 ♂♂ : 255 ♀♀.

Unfortunately the strain died off accidentally on 1964 and only in the summer of 1967 was I given the chance of starting this research again with new specimens of both sexes, which I collected at Marina di Pisa ad nearby. It was not difficult to find again females which even after several copulae kept producing many more females than males in their progeny. I wanted to find out if this characteristic was constant and so I concentrated my observations on a strain derived from female n. 671 (strain "671"); the breeding results for five generations will be given later on in this paper.

Cytological investigations on early embryos of this strain have already shown that their development begins with the haploid of near-haploid chromosome number (Scali, 1968; 1969) and also that later on the chromosome complement is doubled through an anaphasic restitution mechanism. A similar, though not identical, doubling has been described by Pijnacker (1968, 1969) for parthenogenetic embryos. Since the haploid development of the embryo has been a common feature for all thelytokous progenies of mated females (Scali, 1969 and umpublished data), I decided to find out if this was due to a lack of amphimixis or to a failure of sperm penetration (obligatory parthenogenesis).

MATERIALS AND METHODS

The breeding of strain "671" did not require any special care, the specimens being normally vigorous and healthy. Insects were fed on bramble leaves in well aerated standard cages (28×24×26 cm) exposed to a natural photoperiod at 22–24° C.

Eggs were collected at regular intervals, kept in test-tubes 10 × 100 mm in size, at 24–26° C with 70 % relative humidity.

The sex of the larvae was ascertained soon after hatching with the aid of a dissecting microscope through the test-tube wall; it is actually possible to distinguish males from females on the basis of the different abdominal morphology (Scali, 1968).

For cytological investigations, in addition to the eggs of strain "671" I have used control eggs laid by fertilized amphigonic females collected on the Isola del Giglio (Tuscan Archipelago) where the species shows a normal sex-ratio; previous lab breeding of 10 couples at the above mentioned conditions had given the same normal sex-ratio. For each egg to be investigated, the time interval from laying to fixation was exactly established. Fixation occurred in a 3 : 1 alcohol-acetic acid mixture for 6–12 hours after removing the operculum and puncturing the underlying vitelline membrane. To contrast sharply sperm heads with the yolk, the Feulgen reaction was used. The exochorion and the endochorion were completely removed and then the whole egg was hydrolized in HCl 1 N, for 8 minutes at 58° C and kept in Schiff's liquid for 90 minutes at 4° C. A thin slice of yolk roughly corresponding to the micropylar plate was then cut off with a razor, squashed in 45 % acetic acid and mounted by the freezing method in Euparal or Canada Balsam.

RESULTS

A) *Breeding*.

Table I gives the number and the sex of descendents for 21 females of strain "671", for each female several copulae with at least two males were observed and the presence of motile sperms in their genital ducts was ascertained at intervals.

TABLE I

Strain "671": Breeding results for five generations.

Generation	I	II	III	IV	V
	♀ 671(3 : 113)	→ A(1 : 259) ↗	A_1(5 : 298) →	$A_{1,1}$(0 : 401) →	$A_{1,1,1}$(0 : 222)
		↘	A_2(0 : 281) →	$A_{2,1}$(0 : 340) →	$A_{2,1,1}$(1 : 290)
		→ B(0 : 261) →	B_1(0 : 297) →	$B_{1,1}$(2 : 306) →	$B_{1,1,1}$(0 : 381)
		→ C(5 : 204) ↗	C_1(0 : 244) →	$C_{1,1}$(0 : 332) →	$C_{1,1,1}$(0 : 298)
		↘	C_2(0 : 304) ↗	$C_{2,1}$(0 : 274) →	$C_{2,1,1}$(0 : 252)
			↘	$C_{2,1}$(0 : 295) →	$C_{2,2,1}$(0 : 256)

We give in brackets the number of males and females obtained from each mated female of the strain.

From Table I it is clear that all females have had offsprings with females greatly outnumbering males; actually 14 of them produced only females so behaving as obligatory parthenogenetic.

The overall male percentage in each generation decreased from the first generation onward, and in the fourth and fifth it approached that for impaternate males (Bullini, 1966; Scali, 1968). The "671" males looked morphologically normal and those who reached sexual maturity (six in all) fertilized virgin females of bisexual populations as was clear from the normal sex-ratio of the offspring. The possibility of having a few males in the progeny does not seem a constant feature for mated "671" females; in fact females which had "brothers" may or may not produce some males. On the other hand one or two males were again produced by two females ($B_{1,1}$ and $A_{2,1}$ of Table I) after two "all-female" generations. Finally I want to remark that all egg batches had a normal hatching percentage (more than 80); a differential embryonic mortality must therefore be excluded as an important cause for the scarcity or lack of males (see also Scali, 1968).

B) *Cytological investigations.*

Table II gives the result of sperm searching in 32 amphigonic eggs (controls) investigated at different times (1 to 47 hours) after laying: in 16 of them one or more sperm heads were detected.

Sperm heads are found in the outer layers of the egg and are about 13 microns in length (figs. 1–3).

In several instances at one end of the head it was possible to make out a colourless refractive fibre: this suggests that the whole sperm penetrates into the egg. Sperm heads may become wound and undergo pycnosis in eggs which are more than 15 hours old.

In two eggs (marked by an asterisk in Table II) in addition to one sperm head two dividing cells were found. Their exact chromosome number could not be established but it was well above the haploid one and two large methacentric chromosomes could be identified in each cell (see Scali, 1969). We can be reasonably sure that in these eggs amphimixis had already been accomplished by another sperm. If we add these two eggs to the other 9 in which polyspermy has been directly established, we find that 11 out of 16 eggs are polyspermic.

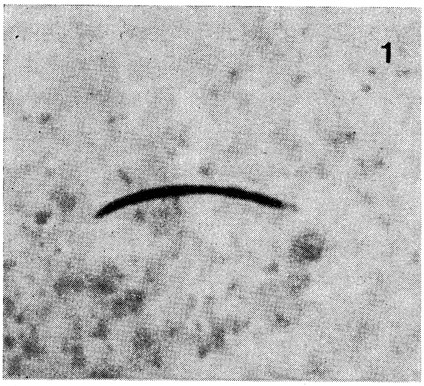
The same investigation was carried out on 72 eggs of strain "671", fixed from 1 to 47 hours after being laid. The eggs were taken in equal number from those laid by the six mated females recorded in Table I as generation V. The result was very different from that of the control eggs: in none of them could sperms be found. Morphological observations of females and eggs of strain "671" did not reveal any significant difference from amphigonic ones.

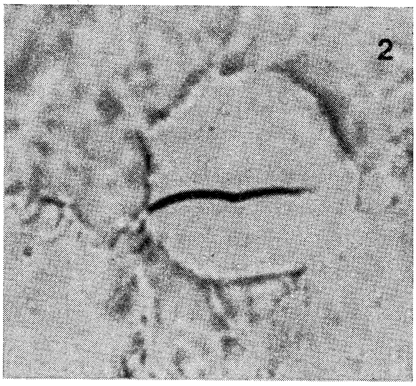
TABLE II

Results of sperm heads search in the micropylar area of 32 amphigonic eggs.

Couple	Number of eggs	Hours from laying	Number of sperm heads per egg
A	2	1	0–0
	1	5	1
	1	11	0
	1	15	2
	1	16	3
	1	20	0
	1	45	1 (*)
B	2	2	0–0
	1	6	1
	1	7	2
	1	11	0
	1	16	2
	1	18	2
	1	47	7
C	1	1	0
	1	4	0
	1	11	1
	2	14	1–0
	1	29	0
	1	39	1
	1	47	1 (*)
D	1	1	1
	1	2	0
	2	12	2–0
	1	14	0
	1	15	0
	2	16	3–0
	Total 32		

(*) In this egg 2 dividing cells have also been found.





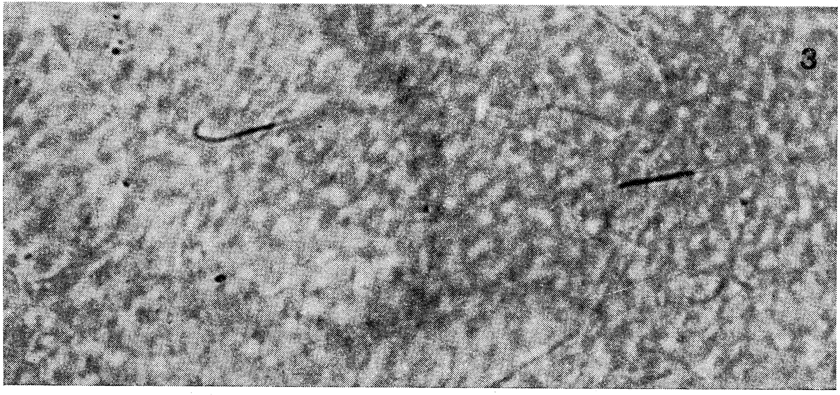


Fig. 1. – Sperm head in an egg fixed 5 hours after laying (×1875).
Fig. 2. – Sperm head in an egg fixed 15 hours after laying (×1875).
Fig. 3. – Two sperm heads found in an egg fixed 12 hours after laying (×910).

DISCUSSION

The above reported results support the existence of obligatory parthenogenetic females already suggested by breeding data (Scali, 1968) and by the haploid development of eggs which should have been normally fertilized (Scali, 1969 and umpublished data).

The complete failure to find sperms in 72 eggs laid by fertilized females of strain "671" cannot be explained by chance, because in one half of the eggs laid fertilized by females of a bisexual population one or more sperm heads were detected with the same method. It has not yet been possible to establish why sperms do not penetrate into the eggs in strain "671".

The fact that only 50% of amphigonic eggs seems to contain sperms is not surprising if we consider the following two points:

1st. The method used presents limitations and technical difficulties which certainly lower the percentage of success. Actually once sperms have got into the egg they scatter and migrate to the ventral side towards the oocyte nucleus; there does not exist then a precise point where to look for them.

2nd. Some eggs can really escape fertilization in spite of the great number of still available sperms (Scali, 1969).

The finding of two or more sperm heads (up to seven) in the same egg definitely proves that polyspermy occurs in *B. rossius*: it seems also that this condition may be quite common. The number of sperms found agrees with that reported for several insect species (see Schröder, 1928); it must be mentioned however that Cappe de Baillon and Vichet (1939) once refer to a « masse » of sperms which penetrates into the egg, without saying though if they have seen it, or if they just say so for the generally accepted polyspermy in heavily lecithal eggs.

The ascertained existence of obligatory parthenogenetic females suggests a new interpretation of breeding results obtained by Cappe de Baillon and Vichet (1939) who crossed a male captured near Montpellier (and very likely of parthenogenetic origin) to parthenogenetic French females. In the progeny only females were obtained and the male was thought to be non functional as is the rule for those odd males which are produced from time to time in unisexual species. However the male used for the cross was morphologically and cytologically normal; since all tested males of *B. rossius* including the impaternate ones (Bullini, 1964; Scali, 1968) have been proved to be thoroughly normal and functional, it seems more likely that the thelytokous progeny was to be ascribed to some anomaly of the females rather than to the male.

In crosses between parthenogenetic females and amphigonic males of some Italian populations (mostly of central Italy) only 10 % of males have been produced (Bullini, 1968); furthermore some couples have had a completely feminine offspring.

Favrelle and Vichet (1937, 1948) showed that no reproductive barrier has been built between French females and North African males. The sex-ratio of the progeny for the 1935 crosses is not clearly recorded (25 ♂♂ obtained from 648 eggs laid by 5 females) and for this reason a partial isolation cannot surely be excluded.

Subsequent crosses, however, produced the two sexes in a normal 1 : 1 ratio. If we compare these data with those for Italian populations it seems that a more effective reproductive barrier exists among closer population than among very distant ones such as French and North African.

To explain the irregular fashion in which bisexual and unisexual populations are often scattered in Central Italy, Bullini (1969) has put forward the hypothesis that some isolated unfertilized females can give rise to parthenogenetic populations and that amphigony can spread into parthenogenetic areas because of the occurrence of functional impaternate males from time to time. To complete this complex picture we must now take into account obligatory parthenogenetic females: they can strongly favour the evolution towards the parthenogenesis of amphigonic populations and/or counteract a possible turn to amphigony (in spite of the occurrence of impaternate functional males) of the parthenogenetic ones.

It will be of great advantage for a better understanding of the reproductive biology of *B. rossius* in nature to establish how many females present a partial or a complete incapacity of egg-fertilization both in bisexual and unisexual populations.

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REFERENCES

[1] CAPPE DE BAILLON P., FAVRELLE M. and DE VICHET G., « Bull. Biol. Fr. Belg. », *71*, 129 (1937).

[2] CAPPE DE BAILLON P., FAVRELLE M. and DE VICHET G., « Bull. Biol. Fr. Belg. », *72*, 1 (1938).

[3] FAVRELLE M. and DE VICHET G., « C. R. Acad. Sci. », *204*, 1899 (1937).

[4] BAEHR W. B. VON, « Zool. Jahrb. Anat. Ontog. », *24*, 175 (1907).

[5] BAEHR W. B. VON, « La Cellule », *30*, 315 (1920).

[6] MONTALENTI G. and FRATINI L., Proceed. XVth Intern. Congr. of Zool., London, 749 (1959).

[7] BENAZZI M. and SCALI V., « Acc. Naz. Lincei, Rend. Sc. fis. mat. e nat. », ser. 8ª, *36*, 311 (1964).

[8] BULLINI L., « Acc. Naz. Lincei, Rend. Sc. fis. mat. e nat. », ser. 8ª, *40*, 926 (1966).

[9] BULLINI L., « La Ric. Sci. », *12*, 1270 (1968).

[10] BULLINI L., « Arch. Bot. Biog. It. », ser. 4ª, *14*, 292 (1969).

[11] SCALI V., « Atti Soc. Tosc. Sc. Nat., Mem. », ser. B, *75*, 108 (1968).

[12] SCALI V., « Acc. Naz. Lincei, Rend. Sc. fis. mat. e nat. », ser. 8ª, *46*, 110 (1969).

[13] PIJNACKER L. P., « Genen an Phaenen », *12*, 129 (1968).

[14] PIJNACKER L. P., « Genetica », *40*, 393 (1969).

[15] SCHRÖDER C., *Handbuch der Entomologie*, Band I, Jena (1928).

[16] CAPPE DE BAILLON P. and DE VICHET G., « C. R. Acad. Sci. », *209*, 525 (1939).

[17] BULLINI L., « Acc. Naz. Lincei, Rend. Sc. fis. mat. e nat. », ser. 8ª, *36*, 898 (1964).

[18] FAVRELLE M. and DE VICHET G., « C. R. Acad. Sc. », *226*, 1108 (1948).