SOME ASPECTS OF THE REPRODUCTIVE BIOLOGY OF Bourletiella (Cassagnaudiella)

PRUINOSA (Tullberg, 1871) (Collembola: Sminthuridae)

E. KLAVER

ABSTRACT
Rearing of Bourletiella pruinosa collected in the Dutch dunes, demonstrated that spermatophora are placed at random without any apparent mating behaviour. The spermatophora do not seem to have any attraction to the females. An aggregational behaviour, which would enhance the meeting of a spermatophore by a female, could not be demonstrated.

The spermatophora are exceptionally resistant to dry and hot air for long periods. They are, contrarily, very sensitive to liquid water, but dew seems not to damage them in nature because they are often deposited on strongly absorbent substrates, notably lichens.

A strong indication is found of the presence of two generations of this species. Females are not sexually neutralized, but attain their secondary sexual characteristics only gradually. The suggestion is made that females with the genital orifice open, but lacking most secondary sexual traits are not reproductively qualified.

INTRODUCTION
Recently, attention was drawn by Ellis, 1975, to the sexual neutralization in Bourletiella pruinosa, occurring in the dunes of the Netherlands. In the genus Bourletiella normally a rather elaborate mating behaviour takes place (Bretfeld, 1970). This seems to be highly functional, since Bourletiellinae generally live in comparatively dry habitats, where spermatophora placed at random, as is usual in Collembola, would have to wait too long before being taken up haphazardly by a female, and dry out. By a mating behaviour this waiting time is reduced to no more than a few seconds. The interest of the sexual neutralization of B. pruinosa
was that, since the morphological prerequisites for a mating behaviour are lacking, there was ample reason to believe that spermatophores would be placed at random, as was also found by Raynal, 1973, in neutralized B. rudula Gisin, 1946 (see also Cassagnau, 1964).

However, the fact that B. prunosa is restricted to a very dry habitat is in apparent contradiction to the functionality of a mating behaviour, as explained above, and it seemed of interest to study in some detail the reproductive biology of this species.

This study was carried out in the Institute of Taxonomic Zoology (Zoölogisch Museum). I am grateful to Drs. W.N. Ellis for his help and advice; the permission given by the board of the Provinciale Waterleiding Duinen, proprietors of the dunes of Castricum, to do the field work in this area, is gratefully acknowledged.

METHODS

Mass cultures were kept in tea-glasses with a diameter of approx. 6 cm, provided with a layer of approx. 3-4 cm of the usual plaster-charcoal mixture. The vessels were closed with a wet sheet of cellophane ("Opecta"), which upon drying has some important advantages over the usual glass cover: the material is crystal clear, and shrinks a little, thus tightly closing the vessel. Cellophane does, to an appreciable extent, not prevent the gas exchange. A natural gradient of air humidity above the rearing medium is thus maintained. Moreover, condensation on the cover, or formation of drops - nefarious in a culture since the animals can be captured in the surface tension - is completely prevented.

Cultures were watered with tap water by means of a syringe which was inserted through the cellophane (the spot of the hole was reinforced beforehand with a small piece of adhesive tape). The hole was loosely closed with a thumb-tack to prevent escapes.

On the plaster some lichen branchlets (Cladonia sect. Cladina) were loosely strewn. This provided the animals with a natural substratum which apparently was preferred above the plaster. It moreover formed a good, and almost not moulding source of food. The Cladonia enabled the animals to choose their preferred place in the moisture gradient.

Solitary specimens were kept in glass tubes of 5 cm long and with a diameter of 2.5 cm, with a layer of approx. 2 cm plaster, and lichen. Individual specimens were kept alive for 4 weeks, specimens in mass cultures for 4-5 weeks.

Cultures were kept at room temperature during daytime, and were placed in a refrigerator at 5°C at night, to simulate as far as possible the strong temperature fluctuations of the natural habitat. (Cultures of Allama fusca (L.), used for some comparisons thrived better when stored overnight in the open.)

All material used was collected in the dunes of the Provinciale Waterleiding Duinen, at Castricum, where the species is locally abundant in dry dune valleys with a low vegetation (Ellis, 1975). In his vegetation map of this area, Do in g, 1965-1966, refers to these vegetation as "pure Koele rion landscapes".

Sexing the specimens alive was difficult; initially only the production of eggs or spermatophores of single specimens could definitely prove their sex, but later a method was developed in which the specimens were etherized (in an apparatus as in use for work with Drosophila), and studied with a magnification of 250-600 times. Females were then marked with a dot of waterproof ink ("Rotring") on their dorsum. After some experience, the recovery rate was quite high. Females are in the mean larger than the males, and have a more bulky appearance. With a certain degree of reliability sexing can also be done using these characteristics.

MATING BEHAVIOUR

To establish whether a specialized mating behaviour would exist or not, the events were recorded when two specimens met each other. Never more than five meetings were studied between one couple. As a meeting was considered the event that two specimens came so close to each other that their distance was less than the length of their antennae in natural position.

In all, 33 meetings were recorded between specimens belonging with absolute certainty to the opposite sex; moreover, 16 meetings were seen between specimens that were, to judge after their appearance, males and females.
From all meetings thus recorded the same picture emerged. The animals, walking erratically, (and/or feeding), happen to touch each other, normally with the antennae, though sometimes with the antennae against other body parts. After some short antenatal movements, not different from those made when an animal stumbles against a lichen branchlet or other object, the animals separate without having shown any mutual interest.

Four times meetings were witnessed between specimens belonging with certainty to the same sex (2 males, 2 females), and another four meetings were recorded between specimens with reasonable certainty belonging to the same sex. In these 8 meetings the behaviour was completely identical to that of the heterosexual meetings.

The general conclusion which emerges is that a sexual attraction or behaviour seems to be lacking altogether. The low total number of meetings recorded (57) also reflects the lack of sexual attraction, since the animals seem to "meet" each other only by pure coincidence during their random walks.

Although no actual case of spermatophore production was seen - this takes place in a very short time in Collembola - it seems reasonable to presume that the spermatophores found in the cultures are placed without accompanying mating behaviour. Moreover, spermatophora were regularly found to be produced by males kept solitarily. These spermatophores, of the normal Smirnichuridae type, were placed at random, but with a very evident preference for lichen branchlets over the plaster substrate. No attempts have been made to count exactly the number of spermatophores produced; males in the reproductive phase seem capable of a daily production of 5-10 spermatophores.

MEETING A SPERMATOPHORE

Actual contact of walking specimens with a spermatophore has been witnessed only in 10 instances. On 4 occasions this concerned specimens of unknown sex (one of them a juvenile); three cases concerned in all probability females, and two other cases certainly females. Only in one occasion a probable male touched a spermatophore.

The last mentioned contact resulted in the head of the spermatophore detaching itself from the stalk; the head remained virtually intact. At a meeting of a female with a spermatophore the head was injured, and some of the contents were smeared over the abdomen. In all other cases the result was an occasional bending of the stalk. Presumably the envelope of the head is rather tough.

The animals did not demonstrate any special behaviour in relation to spermatophora, and the few meetings seen seemed to be the result of a completely random movement. Once in bodily contact with a spermatophore no more special behaviour was shown in particular not any indication was seen of an attempt to make contact between the spermatophore and the postabdomen. Nor did the specimens ever try to eat a spermatophore.

It may thus be presumed that the spermatophores have no special attraction for the females.

AGGREGATION

Given the fact that spermatophores are placed at random and without any sexual behaviour, it seemed not unreasonable to suppose that S. pruinosa would demonstrate a tendency for aggregation. Such, indeed would considerably enhance the chance of a spermatophore to be used before having dried out or otherwise being incapacitated. Moreover, aggregation in Collembola has been demonstrated in a number of instances (Christiansen, 1964; Joosse & Verhoef, 1974, to cite some).

To estimate this possibility in S. pruinosa, mass culture vessels were used. On the plaster surface a netting was scratched with a maze width of about 1/4 cm. To provide the animals with a not too unnatural surrounding, the plaster surface was loosely, and as equally as possible, strewn with Cladonia branchlets. A number of specimens was introduced in the vessels, and these were kept a night in the refrigerator to give the animals the time to accommodate themselves. The day after, every half hour the number of specimens present in each square was counted. Since the counting had to be done rather quickly, and moreover some animals managed to hide themselves between the lichens, the total number on each occasion varied and consequently the mean number per square.

The experiments were done on three occasions (A, B and C). On A only one vessel was used; the mean number of specimens counted (E) varied be-
between 0.66-1.26. B represents two vessels, \( \bar{z} \) ranging in \( B \) from 0.72-1.81, and in \( B \) from 1.28-1.84. C also represents two series, \( C \), with \( \bar{z} \) from 0.44-0.72 and in \( G \) from 0.53-0.69.

As an index of aggregation customarily the quotient of variance/mean is used (a value of 1 indicating random distribution according to the Poisson law, a higher value indicating aggregation and a lower one repulsion). An improvement is the \( I \) of David & Moore, 1954, cit. Pielou, 1969: \( I = s^2/\bar{z} - 1 \); evidently a positive value of \( I \) indicates aggregation. Lloyd, 1967, cit. Pielou, 1969, moreover gives an index of patchiness (\( P \) in table I): \( P = I + I/\bar{z} \).

The significance of both indices can be tested by a chi-square test.

Values of \( \bar{z} \), \( I \), \( P \) and their significance of all series is given in table I.

It is striking that in the same vessel extreme and abrupt changes in \( I \) and \( P \) do occur. Moreover in two vessels with comparable densities aggregational behaviour may differ strongly.

A well-known disadvantage of \( I \) and \( P \) is that they are both strongly dependent on the value of \( \bar{z} \). Lewis & Taylor, 1972, and Cancela da Fonseca, 1966, advocate another approach, starting from the assumption that (within a biologically meaningful range of densities) variance and mean are related by the formula \( s^2 = a \bar{z} \). After transformation \( X = \log \bar{z} \), \( Y = \log s^2 \) and \( Z = \log a \), this means that \( Y = bX + Z \). This can be analysed by elementary statistical means of linear regression.

If this is applied to all observations together, the following values are obtained: \( n = 72 \), \( \bar{z} = 0.0737 \), \( \bar{Y} = 0.0700 \), \( Z = 0.1492 \), \( b = 1.0744 \). Part of sum of squares explained by regression = 2.2871; not explained fraction = 0.9893. Standard deviation = 0.0845, \( z = 12.7212 \) and regression is thus highly significant (\( P < 0.001 \)). The formula is then: \( s^2 = 1.4099 \bar{z} - 1.0744 \). The deviation of \( b \) from 1 is not significant (\( t = (1.0744 - 1)/0.0845 = 0.8805, df = 70 \)).

This means that in the density range tested no indication can be found for an innate aggregational behaviour. The possibility that such a tendency might exist at considerable lower densities can not be dismissed but could not be tested. It is, however, questionable whether aggregation at these low densities would have much direct influence on efficient use of spermatophores.

### SOME ASPECTS OF THE SPERMATOPHORA

Once it became apparent that the spermatophora are not produced in mating behaviour, and thus have to stand a more or less prolonged period of dessication, it seemed interesting to study some details of the resistance of the spermatophora against adverse conditions.

For purposes of comparison, the spermatophora of *Allacma fusa* (L.), were used. This species, which could be reared with ease, is common in the broad-leaved woods in the dunes, and is usually encountered on rotting branches lying on the ground. This species profusely produced spermatophora, in a much higher number than *B. pruinosa*.

The spermatophora of *B. pruinosa* are comparable in shape to those of *A. fusa* which are described in more detail by Mayer, 1957, and Schlima, 1965. The head is nearly globular, with a diameter of approx. 35-43 \( \mu \).

a) Temperature - dryness resistance of spermatophora.

It soon became apparent that the spermatophora of *B. pruinosa* are almost insensitive to dry air or heat. Spermatophora kept as long as twelve days at room conditions remained completely unaltered. Neither the dimensions of the head (as seen with a stereomicroscope) nor the crystal clarity of the contents showed any change. An illumination with two focussed microscope lamps during 50 minutes did not provoke any change. The same treatment to *Allacma* spermatophores made them begin to shrivel after about 15 seconds, and after half a minute they were completely shriveled. The diameter changed in that short period from 120 \( \mu \) to 50 \( \mu \). To comprehend the extraordinary resistance of the tiny sperm droplets against water loss, one must realize that the relative surface of a drop with a diameter between 35 and 43 \( \mu \) is the same as that of a liter of liquid, poured on a surface of 140-170 m².

It has been attempted to measure an eventual swelling of the head in high air humidities, but results were beyond the resolution power of the stereomicroscope at 80 x.

b) Experiments with osmosis.

Schlima, 1965, gives a detailed account of treatment of spermatophora of *A. fusa* with various...
### TABLE I

Results of experiments on aggregation, repeated at three instances (A, B, C), the last two times in twofold. Each time counts were made at intervals of half an hour; presented are the mean value per square, the values of I and P and their eventual significance, * indicating a probability of occurrence between 0.05 and 0.01, ** a probability between 0.01 and 0.001, and *** a probability smaller than 0.001.

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sucrose solutions. He found that in distilled water the spermatophora burst in about 30 seconds, in a 0.02% solution after 35.5 seconds, in a 0.05% solution after 57 seconds, in a 0.1% solution after 134 seconds, in a 0.2% after some hours, and in a 0.5% solution after some days.

To check the possibility that the superior drought resistance of *B. pruinosa* spermatophora might be due to a higher osmotic value of its contents, the experiments of Schliwa were repeated for *pruinosa* and *fusca* as a control.

Unfortunately, the results of Schliwa in *A. fusca* could not be reproduced.

Bringing spermatophora of no more than 24 hours age in contact with solutions of 0, 0.05, 0.2 and 0.5% made them burst invariably within 10 seconds. In concentrations of 1 and 2% the survival time was about 30 seconds. Treatment with a 5% solution made the head burst after ± 1 minute, the remains kept a spherical shape, though swollen 2-3 times their former size.

The differences with the observations of Schliwa are striking; an explanation may be found in the different age of the spermatophora used. Schliwa did not specify this, although he remarks that old spermatophora have a higher suction value.

Spermatophora of *pruinosa*, not older than 30 hours, brought into contact with solutions of 0, 0.05, 1 and 5% sucrose behave identically: the head was immediately detached from the stalk, the membrane disappeared within some seconds and the head desintegrated, though the remains more or less kept a spherical shape. In a 10% solution the head desintegrated as well, but was not detached from the stalk. The general appearance is that the spermatophora are destructed by contact with any liquid suggests that rain, and dewdrops, might be destructive for the spermatophora in field conditions. In this connection it is interesting to note that in early morning, when the phanerogamous vegetation is wet by dew, no dewdrops are present on the *Cladonia*; evidently the dew is absorbed in the lichens before it can form droplets on its surface.

c) The membrane of the head.

On some occasions it could be observed that the membrane of the spermatophore head became fragmented, but did not dissolve. This suggests a membrane consisting of a denaturalized protein, instead of a simple "condensation-membrane", as suggested by Schliwa.

**SOME DETAILS ON DEVELOPMENT AND PHENOTYPIC NEUTRALIZATION**

To follow the development of a population, a sample was collected ten times at a given locality near Castricum (the "Gevers Duin"), where a strong colony exists. As far as the unfavorable weather conditions permitted collections were made with intervals of about two weeks. Material was sorted in males, females and immatures (genital papilla closed). Females were divided into three categories:

a) only a few setae on the genital papilla; appendices anales and broadened perianal setae not differentiated,

b) genital papilla with normal number of setae, appendices anales and broadened perianal setae recognizable, though not strongly developed, perianal setae not lacerated,

c) as b but perianal setae strongly lacerated, appendices anales well developed.

The results are summarized in fig. 1.

On top of the diagram the total numbers captured are indicated.

The first 4 collections were made by making 20 sweeps; after middle August the population density declined strongly, and 30 sweeps were hardly sufficient to obtain a workable sample. The decline was caused doubtless by an exceptionally prolonged and heavy rain period, that continued throughout the following month. It is unfortunate that the population development may thus be not quite normal in some details. The two specimens collected on November 5th were the results of 60 sweeps.

All specimens counted were passed under the microscope, and in all 299 males studied closely not a trace of a secondary sexual structure was apparent. Nor was there a reason to presume this in any of the hundreds of specimens that I kept in culture, and studied at low power.

The first individuals were seen on June 5th, though only a small number could be collected (18). This first sample consisted of males, immatures and type females. From that day onwards the number of males declined at first (but perhaps was the high proportion of males in the first sample a coincidence caused by the small sample size) and
then rose, then remained almost at the same level. The proportion of "young females" (group a) predominated, to make place later for females of type b and finally of type c.

From the second half of August a second generation started (contrary to Ellis, 1975). Unfortunately the aberrant weather conditions and somewhat low number of specimens collected did not allow to analyse this in much detail, although the same general picture as in the first generation was evident.

It is interesting to note that in a mass culture set up with specimens from June 5th after two days the first spermatophores were found. The first eggs however, were found on July 7th, in a solitary rearing that was started on June 26th, while the first eggs in a mass culture, started on June 12th, were found on July 19th.

The data on the various female types confirm the conclusion by Ellis, 1975, that the females do not show a true sexual neutralization but obtain their complete set of sexual traits only after some delay. Although no special study was devoted to egg-laying, in one occasion I could observe this in some detail: walking slowly backwards, and touching now and then the substrate with her postabdomen, a female ate continuously from the substrate of plaster + charcoal. Suddenly she stopped this behaviour, and an egg was protruded from the genital papilla. While an, initially transparent, thin layer of faeces was deposited on the egg, this was kept in a rotating movement. The anal appendages were constantly moving and appearing responsible for the rotation. After having covered the egg, the female discontinued the rotation. She remained 20-30 seconds with the egg attached to her genital orifice; the covering layer dried, as was evident from its colour changing to that of the dry substratum. Finally the postabdomen was brought down, the egg was placed on the substrate, and the female walked away. In the dry coating the three anal valves had left a highly distinctive pattern. (Although actual egg laying as described above was seen only once, the typical trilobed impression on the egg covering was evident in almost all eggs observed.)

The eggs are placed solitary, sometimes in little clumps, up to six eggs. Eggs are deposited preferably in small crevices, etc.

The, admittedly single, observation of the function of the anal appendages casts considerable doubt on the reproductive value of type α females. Massoud & Pinot, 1973, demonstrated that in Arthropalitidae, belonging to another subfamily of the Sminthuridae, the anal appendages have the same function as described above in covering the eggs in a protective layer of faecal matter.

It is reasonable to presume that the incompletely developed females of pruinosa lacking differentiated anal appendages are not reproductively qualified.

CONCLUSIONS

It seems that the adaptation of Bourletiella pruinosa to its dry habitat is resident in the exceptional drought resistance of its spermatophore head. Presently it is not known, in how far such resistance in spermatophora is shared with other species of dry habitats, as e.g. Sminthurus nigromaculatus Tullberg, or even Entomobrya multifasciata (Tullberg). Neither is it known whether this adaptation may be typical for the subgenus Cassagnaudiella, and thus be shared by B. radula Gisin, and B. pietilium Gisin.

REFERENCES

MAYER, H., 1957. Zur Biologie und Ethologie ein-
Fig. 1. Follow-up in a population of *B. pruinosa* at a certain locality in Castricum, during the summer of 1974: cumulative percentages of juveniles, three types of females and males. Number of specimens on which percentages are based are given on top of the diagram.

Requests for exchange or sale of this publication should be addressed to the Administration of the Zoological Museum of the University of Amsterdam.