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The Effect of Temperature on Populations of Amoebae in Different Biotopes

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Biologia. — *The Effect of Temperature on Populations of Amoebae in Different Biotopes* (*). Nota di MARISA CIGADA LEONARDI, SILVANA CANTALLES e UMBERTO FASCIO, presentata (**) dal Socio S. RANZI.

RIASSUNTO, — Sono state prese in esame quattro popolazioni di amebe di acqua dolce: *Mayorella spumosa* di pianura selvatica, *M. spumosa* di pianura in coltura da decine di anni, *M. spumosa* di montagna (1.800 m) e *M. lyncaea* raccolta in Uganda. Le amebe dei differenti biotopi sono state acclimatate a differenti temperature: 4 °C, 10 °C, 18 °C, 25 °C e poi si è calcolato (in ore) il differente tasso di riproduzione dei 16 allevamenti, nelle differenti stagioni dell'anno, per vedere fino a che punto origine, stagione e temperatura interferissero.

La provenienza della popolazione si fa sentire e anche la stagione ha la sua influenza. Questi due fattori influenzano la velocità di riproduzione alle differenti temperature. Le temperature in alcuni casi appaiono limitanti; tutti i ceppi risentono le basse temperature molto di più in autunno che in inverno.

Le due popolazioni di pianura selvatica e di allevamento in laboratorio appaiono comportarsi in maniera molto simile.

La popolazione di montagna in autunno mal tollera i 25 °C e a 4 °C non è più in grado di riprodursi. Anche *M. lyncaea* ha un limite di tolleranza ristretto in autunno e, a 25 °C, solo in inverno riesce ad aumentare il ritmo riproduttivo.

Le amebe di tutte le popolazioni appaiono possedere un caratteristico ritmo di riproduzione corrispondente anche alle differenti stagioni e questo ritmo sembra fissato nel loro patrimonio ereditario.

During the biological study of fresh water amoebae, carried out particularly on *Mayorella* (*Amoeba*) *spumosa* (Gruber) it was possible to identify differences in the seasonal cycles related to different living environments. The populations in different biotopes vary in size in the course of the year according to the climatic conditions of the environment. It was noted that specimens from plain areas showed an increase in size in spring and in autumn, whereas those of mountain areas increased in size only in the summer [4].

The rate of reproduction also increased in the same periods, from which we can affirm that the biomass of two specimens varies during the year with increases which correspond to the periods in which the environmental conditions are most favourable. The temperature appears to be an important factor influencing these seasonal rhythms.

(*) Researches carried out in the Dipartimento di Biologia dell'Università degli Studi di Milano, with a contribution of the « Accademia Nazionale dei Lincei ».

(**) Nella seduta del 20 giugno 1986.

The same research was conducted on plain and mountain amoebae cultured for some years in the laboratory, in constant environmental conditions: kept in a chamber thermostatically controlled at 18 °C, continuous diffused light, culture medium: infusion of hay at pH 7.4. These amoebae maintain the seasonal rhythms of the original populations. This proves that the characters are hereditary.

A study carried out on amoebae of the *Mayorella* genus, collected at Kampala (Uganda), showed that the biomass of these amoebae, living in an environment in which the temperature does not vary to any considerable extent, changes very little in the different months of the year [3].

Morphological and physiological differences in these tropical amoebae of the *Mayorella* genus have led to their being classified as belonging to a different species: *Mayorella lyncaea* (Leonardi Cigada) [2].

Finley, who studied the rate of reproduction of 10 species of Ciliates [5] at three different temperatures, pointed out the different behaviour patterns of the different species.

It seemed interesting to carry out an experimental investigation to find out just what would be the reproduction rate of the amoebae of different biotopes, after a suitable period of acclimatization at different experimental temperatures. The response of the various populations in the different seasons was studied in order to see the interaction of the culture temperature factor with the seasonal and genetic factors.

Materials and Methods

Experiments were carried out on four different populations of amoebae: plain *M. spumosa* (wild specimens), plain *M. spumosa* (specimens in culture), mountain *M. spumosa* (specimens in culture), *M. lyncaea* (specimens in culture). The wild forms, immediately after collection, were put in culture medium and then used for the experiments. All the culture strains were kept in the laboratory for at least several hundred generations (the plain amoebae have been in culture for decades) in constant environmental conditions, the same for all the strains. The method was described by Leonardi Cigada (1979). The temperatures chosen were: 4 °C; 10 °C; 18 °C; 25 °C. The experiments were carried out in spring, summer, autumn and winter.

The index used to express the reproduction rate is the doubling time (Odum [6]):

$$T = \log_e 2/r \qquad \text{where} \quad r = \Delta N/N\Delta t$$

where T is the doubling time (in hours); r is the specific growth velocity when the growth of the population is exponential, N the number of individuals at the beginning and t the time in which the experiment took place.

The amoebae of each strain were isolated in hanging drop glasses in the culture medium, acclimatized in thermostat at the chosen temperature for a period of 5 days and then were counted. Ten glasses were prepared for each strain, and each temperature, the number of amoebae of each glass was counted for 6 days and then 5 glasses were selected for each strain.

Results

The rate of division observed, keeping the amoebae at different temperatures (Table I and fig. 1), shows a correlation between temperature and reproductive rhythm.

Table I.

Cultures of Mayorella: Reproduction rate (in hours) at different temperatures, in different seasons.

Strains	Spring	Summer	Autumn	Winter
Cultures at 18 °C				
Plain-culture	38	33	26	39
Plain-wild	34	33	24	68
Mountain	32	42	24	39
M. lyncaea	36	34	34	56
Cultures at 25 °C				
Plain-culture	19	33	19	21
Plain-wild.	33	24	26	31
Mountain	25	42	556	18
M. lyncaea	70	69	51	47
Cultures at 10 °C				
Plain-culture	34	35	37	94
Plain-wild.	56	59	62	87
Mountain	36	40	74	45
M. lyncaea	39	49	239	74
Cultures at 4 °C				
Plain-culture	60	45	121	493
Plain-wild.	39	59	98	113
Mountain	78	55	F.	186
M. lyncaea	53	39	F.	144

F. = block of reproduction

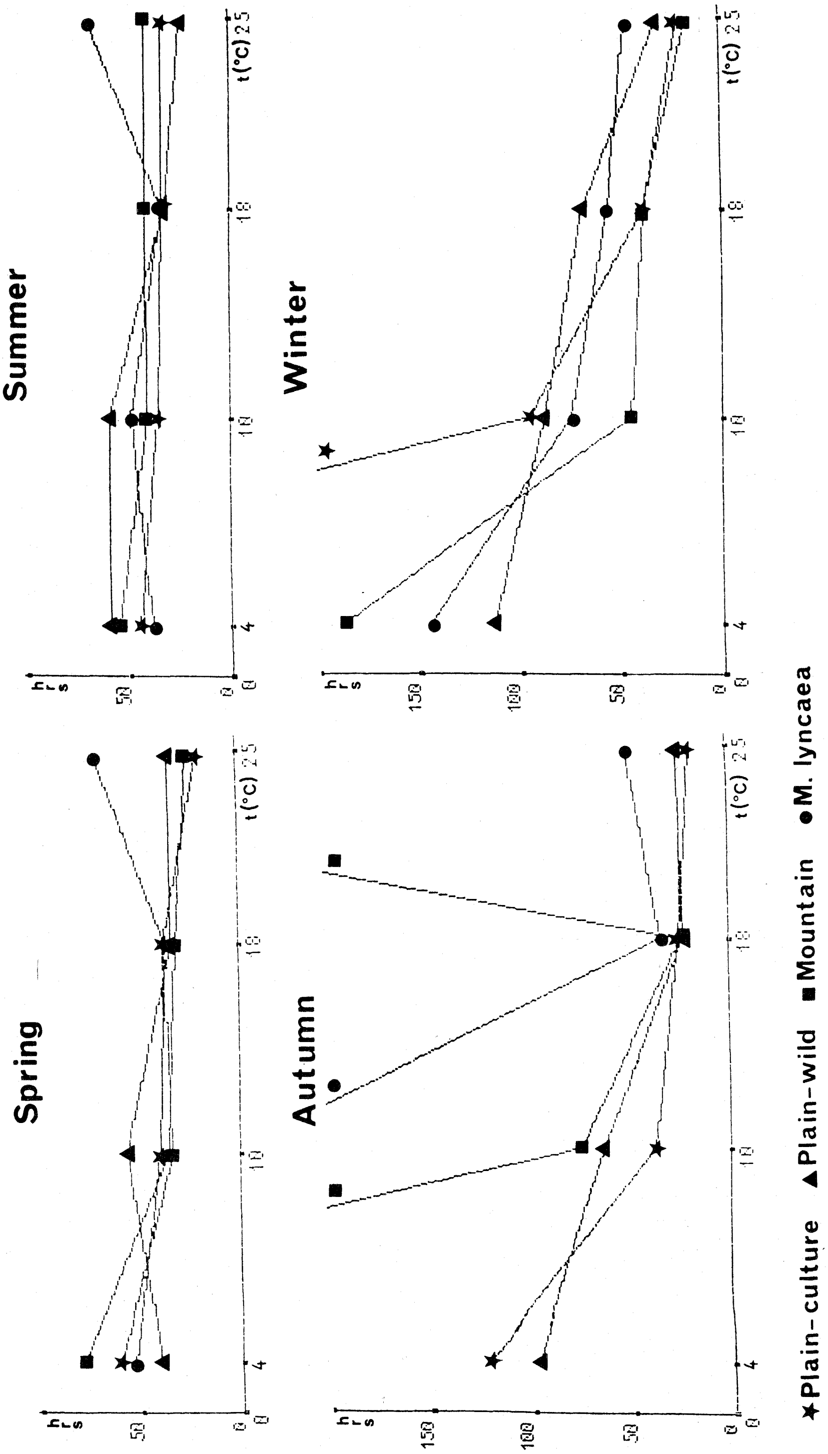

Fig. 1. – *Mayorella*: different strains. Doubling times (in hours) at different temperatures.

Acclimatization at 18 °C for a week in all the populations, led to a stabilized reproduction rate ranging between: 32-38 hours in spring, 33-42 hours in summer, 24-34 hours in autumn, 39-68 hours in winter. It is interesting to note that at such a temperature all the strains reach maximum reproductive rate in autumn: 24-26 hours for all the *M. spumosa* strains, 34 hours *M. lyncaea*. The remarkable autumnal velocity is followed by a slower winter rate: *M. spumosa* 39 hours in plain strain in culture, 68 hours in wild plain strain, 39 hours those of the mountain strain, 56 hours *M. lyncaea*.

In all the strain of *M. spumosa*, acclimatization at 25 °C normally determines a particularly marked increase in the reproductive rhythm in winter, and a barely perceptible increase in spring and summer. The temperature of 25 °C proves to be well tolerated in every season by the plain populations both wild or in laboratory culture. The mountain populations do not tolerate acclimatization at the temperature in autumn.

The exixtence of parallel behaviour emerges therefore in the case of wild and culture *M. spumosa* of the plain; a different behaviour pattern is observed in the case of the mountain population which has considerably narrower limits of tolerance in autumn, reaching very high doubling times.

At 25 °C *M. lyncaea* has a behaviour pattern different from that of *M. spumosa*, slowing down the reproductive rhythm in all seasons except winter.

At a temperature of 10 °C all the strains have short doubling times in spring, keeping to practically the same values observed at 18 °C. This temperature begins to be limiting for amoebae of Uganda in the autumn and for plains specimens in winter.

The slowing down at the lowest temperature (4 °C) is very marked in all strains, and in the case of mountain *M. spumosa* and *M. lyncaea* leads to a total block of reproduction in the autumn.

The lowest doubling times correspond to: 19 hours for plain *M. spumosa* in culture (24 °C in autumn), 24 hours for wild *M. spumosa* (18 °C in autumn), 18 hours for mountain *M. spumosa* (25 °C in winter) and 34 hours for *M. lyncaea* (18 °C in summer).

CONCLUSIONS

There is a clear correlation between temperature and doubling time which in the case of *M. spumosa* diminishes with an increase in temperature.

M. lyncaea on the other hand, has a lower reproduction rate at 25° than at 18 °C, except in winter, which shows that this species has more restricted limits of tolerance at higher temperatures.

No particular variation in the reproductive rate at the different temperatures experimented was observed in spring and summer, but in autumn and winter the temperature/doubling time correlation is extremely evident.

In autumn the mountain population poorly tolerates any increase or decrease in temperature. On the other hand the researches conducted on the seasonal variations of mountain amoebae showed a diminution in both the productive rhythm and the sizes of the individuals in the passage from summer to autumn [3, 4]. At this time, which is critical for mountain populations, the experimental variations in temperature are poorly tolerated and the amoebae slow down their reproductive rate to the extent of almost stopping entirely.

Parallel behaviour is observed in the case of the amoebae of Uganda: the seasonal cycle of this population, while not undergoing particular variation, shows a diminutions of the biomass in the autumn months [2].

But the population of *Mayorella spumosa* of the plain enjoys favourable conditions in the autumn months and the biomass increases. In this present research only the limiting temperature of 4 °C provokes a strong slowing down.

Thus there is a clear interaction of the temperature factor with the season; this factor undoubtedly has a very considerable influence and would appear to indicate the existence of a seasonal biological clock.

The similarity of behaviour between wild *M. spumosa* of the plain and those in culture for thousands of generations, and the diversification of the responses to different temperatures by the mountain specimens to a lesser extent, and by the Ugandan forms to a more accentuated extent, confirms that the data obtained in the preceding researches on seasonal cycles of these amoebae, belonging to different biotopes, are not only under the influence of factors connected with the environment, but are also inscribed in their hereditary characters.

Questions of Systematics

In our first works published on fresh water amoebae the generic name of *Mayorella* introduced by Shaeffer [8] was not used for the small amoebae which move with defined orientation.

Today, taking into account the work of Bovee [1] and Page [7], it would seem better to adhere to the breaking up of the genus *Amoeba* and attribute the amoebae described in the preceding works, the specimens most diffused in fresh water in Lombardy, to the genus *Mayorella*: *Mayorella* (= *Amoeba*) *spumosa* (Gruber) and consequently attribute to the genus *Mayorella* the species studied by me: *Mayorella* (= *Amoeba*) *lyncaea* (Leonardi Cigada [2]).

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