THE BIOLOGY OF ENTOMOBRYOIDES PURPURASCENS

J.E.R. Stainer

ABSTRACT

The biology of Entomobryoides purpurascens (Packard, 1873) (Collembola: Entomobryidae)

M.Sc. Thesis: John E.R.Stainer, Department of Entomology

Certain aspects of the biology of Entomobryoides purpurascens were examined in the laboratory and in the field. This species can be reared under the usual conditions demanded by Collembola but requires, in addition, a source of light. In the field, the life cycle is at least one year in length and includes a growth arrest in which adult animals may lose their external sexual characters. Oviposition takes place from late May to early September, the only part of the year in which mature males are present. The natural diet of E. purpurascens consists of fungal spores and mycelium, pollen and collembolan remains. Evidence is presented that measurements are of no value in the separation of the instars of this species. It is further shown that some plastics and mold inhibiting chemicals may retard or totally inhibit growth.

Jer Stainer

Department of Entomology

Master of Science

THE BIOLOGY OF ENTOMOBRYOIDES PURPURASCENS (PACKARD, 1873)(COLLEMBOLA: ENTOMOBRYIDAE)

by

John Evelyn Randall Stainer

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Department of Entomology
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Montreal.

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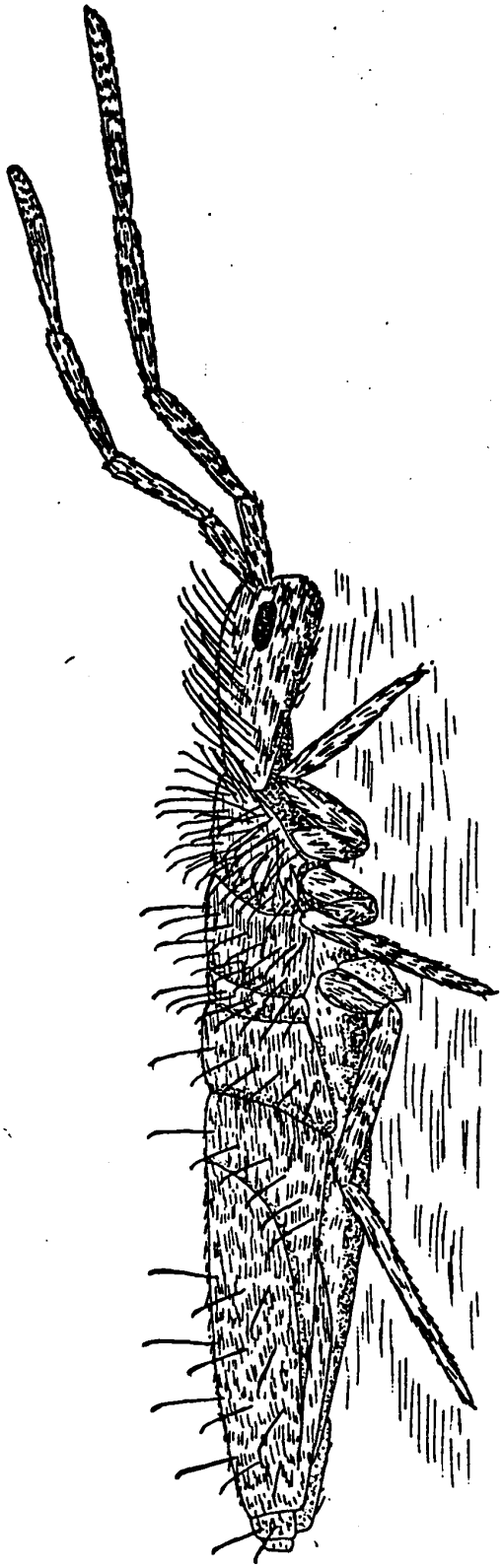
Frontispiece

Entomobryoides purpurascens in normal resting position.



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A research project is seldom the work of one person alone: contributions, conscious and unconscious, are made by all one's friends and associates.

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INDEX

Acknowledgements 1
Index ii
List of Tables iv
List of Figures vi
Introduction 1
Literature Review 3
The Species Studied 3
Requirements and Techniques for Rearing Collembola . 5
Life Histories of Collembola. 12
Diapause 18
Materials and Methods 20
Collection 20
Rearing 21
Equipment 21
Development of Rearing Techniques 23
Demonstration of Diapause 25
Mold Control and Anaesthetics. 25
Studies of Preserved Materials 26
Measurements 26
Proportion of Males and Sexual Maturity 28
Gut Contents 29
Field Observations 29
Specifications and Sources of Supply 30

Results 32
Rearing 32
Studies of Preserved Materials 35
Measurements 35
Sex Proportion Studies 35
Gut Contents 35
Field Studies 36
Discussion 38
Summary and Conclusion 47
References 49
Tables
Figures

LIST OF TABLES

I. A comparison of different rearing techniques.

II. Rearing results and survival records for groups of Entomobryoides purpurascens used to demonstrate the existence of a diapause in the species.

III. Duration of stages of Entomobryoides purpurascens reared in the laboratory at 18°C. based on observation every 24 hours.

IV. Duration of stages of Entomobryoides purpurascens reared in the laboratory at 25°C. based on observations every 24 hours.

V. Ranges and average measurements of lengths of indicated structures from samples of Entomobryoides purpurascens reared in the laboratory at 18°C.

VI. Ranges and average measurements of lengths of indicated structures from samples of Entomobryoides purpurascens reared in the laboratory at 25°C.

VII. Lengths of third femora of male Entomobryoides purpurascens collected from the Morgan Arboretum, Macdonald College, on indicated dates.

VIII. Lengths of third femora of Entomobryoides purpurascens reared in an incubator at 18°C. from 13.IX.67 to 23.XII.67

IX. Gut contents of samples of Entomobryoides purpurascens collected in the Morgan Arboretum, Macdonald College, in 1967.

X. Gut contents of samples of Entomobryoides purpurascens collected in the Morgan Arboretum, Macdonald College, in 1968.

XI. Gut contents of samples of Entomobryoides purpurascens collected in the Morgan Arboretum, Macdonald College, in 1969.

XII. Percentage mature males in samples of Entomobryoides purpurascens collected in the Morgan Arboretum, Macdonald College, in 1966-67.

XIII. Percentage mature males in samples of Entomobryoides purpurascens collected in the Morgan Arboretum, Macdonald College, in 1968.

XIV. Percentage mature males in samples of Entomobryoides purpurascens collected in the Morgan Arboretum, Macdonald College, in 1969.

XV. Temperatures in the habitat of Entomobryoides purpurascens recorded in spot checks during 1968 (in the Morgan Arboretum, Macdonald College).

LIST OF FIGURES

Frontispiece: Entomobryoides purpurascens in normal resting position.

1. Entomobryoides purpurascens: frequency distribution of lengths of antennal segment II.

2. Entomobryoides purpurascens: frequency distribution of lengths of antennal segment III.

3. Entomobryoides purpurascens: frequency distribution of lengths of antennal segment IV.

4. Entomobryoides purpurascens: frequency distribution of lengths of femur I.

5. Entomobryoides purpurascens: frequency distribution of lengths of tibiotarsus I.

6. Entomobryoides purpurascens: frequency distribution of lengths of femur III.

7. Entomobryoides purpurascens: frequency distribution of lengths of tibiotarsus III.

8. Entomobryoides purpurascens: frequency distribution of lengths of unguifer III.

9. Entomobryoides purpurascens: frequency distribution of lengths of unguis III.

10. Percentage of mature males in samples of Entomobryoides purpurascens collected in the Morgan Arboretum, Macdonald College, in 1968.

11. A comparison of spring changes in percentage of males in samples of Entomobryoides purpurascens collected in the Morgan Arboretum, Macdonald College, in 1968 and 1969

12. Interval between collection and oviposition when Entomobryoides purpurascens reared at 25°C. and $15^{3}/4$ hour photophase from date of collection.

13. Egg of Entomobryoides purpurascens showing one half of the chorion and the characteristic ridges in the serosal membrane.

I. INTRODUCTION

The study of Collembola is almost in its infancy. Due in part to their small size, but probably more to their general lack of recognized economic importance, the Collembola have received comparatively little attention from biologists. Few good taxonomic studies have been made, rendering the positive identification of many of these animals difficult, and it is quite possible that the valid separation of some of the smaller species is beyond the capabilities of the light microscope.

This severely limits the scope of any ecological study, for any particular species under special consideration must be identifiable with certainty. While this requirement may be fulfilled, any survey of the other Collembola of the biocoenose will undoubtedly be hampered, in most parts of the world, by a lack of adequate keys and other taxonomic information.

The Collembola present many other difficulties to the ecologist. In general they live in secluded habitats such as the soil or under bark, which render observation and the meaningful measurement of environmental conditions arduous, if not impossible. Collembola continue to molt after maturity, and there is often no way in which to separate living adults from sub-adults. Sexual dimorphism is extremely limited

and in many species it is only rarely if ever that it is possible to distinguish living males from females. Few observations have been made of the fertilization of eggs and the exact mechanism is, in many cases, in doubt.

As a result of these difficulties, any study of the biology of the Collembola must involve much more indirect evidence than would be acceptable when working with other, more convenient organisms. Estimates and subjective observations must be employed because of a lack of instrumentation which will give meaningful, precise information in the situation at hand. It follows that there is always a small chance of error, for while many lines of evidence may point to a conclusion, probably none of them will ever provide conclusive proof.

Entomobryoides purpurascens (Packard, 1873) was chosen for this study because it is large, easy to identify, and available in large numbers, and also because next to nothing is known of its biology. The animal was found to be abundant in woodpiles in the area in which the study took place and could not easily be confused with any of the other Collembola in this habitat. It was found to display a growth arrest which has considerable importance in the life history and rearing of the species.

II. LITERATURE REVIEW

THE SPECIES STUDIED

Entomobryoides purpurascens was described by Packard (1873) who assigned it to the genus Degeeria Nicolet (1842). Ten years later the animal was transferred to the genus Entomobrya Rondani, 1861. More recently, Maynard (1951) erected the subgenus Entomobryoides of Entomobrya, designating E. purpurascens as the type species. Finally, Christiansen (1958) elevated Entomobryoides to the status of genus, which it still retains.

E. purpurascens has two distinct forms (Christiansen, op.cit.), one northern and the other southern in distribution. The representatives here are of the northern form which is found in the northeastern United States and southeastern Canada and, occasionally, on the Pacific coast (Christiansen, op.cit.).

The original description of the species, by Packard (1873), was very brief, but the following redescription of the northern form, by Christiansen (op.cit.) is sufficiently detailed to obviate the necessity of any independent account.

"ENTOMOBRYOIDES PURPURASCENS (Northern form-A)
Color and pattern. Background color white, pigment bluish purple. Pigment slightly darker on posterior segments. Pale, double, V-shaped mark on dorsum of head, two thin dark lines running ventrally from median ventral margin of eyepatch to

venter of head. Antennae slightly darkened. Furcula and base pale, remainder of body uniformly and moderately pigmented.

Antenna. Shape of antennae typical. Third segment with normal paired rods. No accessory pegs were observed. Apical sensory organ of second segment with three minute rods, largest similar in shape and half as large as those of third segment, the smallest about one-third as large as the largest. Smooth setae of first antennal segment absent from apical one-fifth of segment. First antennal segment with numerous short clavate setae, similar to body setae of type one, but about half as large.

Head. Slightly longer than broad. Labral papillae conical, unisetaceous or bisetaceous, with setae small but distinct. Labial appendages with typical number of papillae; external differentiated seta broad, tapered from base, more strikingly so at extreme apex, slightly curved, exceeding apex of same papilla for from one-fifth to one-third of its length. Eyepatches black, trapezoidal, with inner posterior angle acute, about as wide as antennal diameter. Two anterior eyes on either side slightly larger than the two inner posterior ones, which are slightly smaller than the remaining four.

Clothing. Setae of type one normal, slightly curved for entire length, those on anterior body segments sharply bent at expanded apical portion, numerous on all segments

save abdominal six. Setae of type two scarce, small in size, never as long as longest setae of type one. Setae of type five slender, gradually tapered from base, coarsely unilaterally ciliate for apical one-half to two-thirds of length.

Legs. Clothing typical, finely ciliated setae on tibiotarsus slightly larger than normal setae of same segment. Trochanteral organ typical, with many setae, those in arms extremely long, strikingly graduated toward apical seta. Internal setae usually about one-third as long as apical seta. Unguis normal, with usual seven teeth moderate in size, apical internal tooth about half as large as others, which are subequal. Basal internals slightly above mid-level of internal edge, laterals on a level with basal internals, and external tooth. Empodial appendage acuminate, heavily ciliate along internal edge.

Furcula and genital plate. Anteapical tooth of mucro subequal to apical. Apical strongly curved, anteapical erect. Basal spine exceeding apex of anteapical tooth. Genital plate with sixteen acuminate setae; basal pair gradually tapered and slightly curved, slightly larger than remaining setae which are strongly angulate."

REQUIREMENTS AND TECHNIQUES FOR REARING COLLEMBOLA

It is generally accepted that Collembola and other soil arthropods require a high humidity and the needs of Collembola in this respect were studied by Davies (1928)

who found that, while all other species tested normally required high humidity, Entomobrya multifasciata [Brook] [=E. nivalis (Linnaeus)] could tolerate lower relative humidities. Davies extended the results to imply that the entire genus had similar requirements. Davis and Harris (1936), on the other hand, showed that Pseudosinella violenta (Folsom), in all stages, required a one hundred per cent. relative humidity in order to thrive.

The maintenance of suitable humidity conditions is closely interrelated with the choice of substrate. The rearing equipment should control the moisture level without involving the researcher with inconveniently frequent maintenance. Macnamara (1924) reared his animals on rotting wood in small glass vials and Thomas (1929) used a sterile straw-manure mixture which had been seeded with mushroom spawn. Neither of these methods, however, permitted direct observation of the Collembola. Britt (1951) made use of damp filter paper, but this method required constant maintenance. Uchida and Chiba (1958) used filter paper over sand, but the most successful substrate is plaster of paris, to which is usually added 10 per cent. by volume of activated charcoal, which renders the animals more readily visible. Filinger (1928), rearing centipedes, Jenkins (1947), rearing mites, and other workers rearing Collembola (Willson, 1960; Marshall and Kevan, 1962; Green, 1964a, Vail 1965; and others)

have used such a mixture, while Lindenmann (1950) omitted the charcoal. Rohde (1956) described in detail a rearing technique using plaster-charcoal and Huber (1958) assessed the use of the color of this mixture as an indicator of the humidity of the rearing containers. When precise control of humidity is needed, varying concentrations of sulfuric acid (Davies, 1928) or of salts (Doane and Allan, 1968) may be used. Winston and Bates (1960) include in their publication an extensive list of the literature on this means of humidity control.

With the exception of Doane's apparatus, all of the rearing apparatus listed above involves the use of closed containers without air circulation. Other more complex containers have been devised and some are commercially available, but these do not appear to have been used for the rearing of Collembola, probably because of their high cost.

With regard to the rearing containers themselves, it has been shown by Chada (1962) that some plastics can affect the rearing of insects in the laboratory. Greenbugs (Toxoptera graminum (Rondani)) and young barley plants were adversely affected by cages of cellulose acetate and vinyl plastic but not affected by cellulose nitrate. The toxic materials resulted in the death of the plants within ten days. The greenbugs showed reduced fecundity and generally retarded development. Chada cites other work describing

the toxicity of certain plastics to plants and fish. This possibility of toxicity of the materials used must therefore be taken into consideration in the design of rearing equipment.

Very little work has been done on the light requirements of Collembola and that which has been done lacks precision. Few researchers even mention the light conditions under which they reared Collembola. Bellinger (1954) states that there has been no extensive comparative study of the factor. Neither Lindenmann (1950) nor Milne (1960) mention light requirements. It is interesting to note, however, that the two species which Milne was unable to rear were Isotoma viridis Bourlet and Neanura muscorum (Templeton), both of which are surface dwelling forms and could concievably have a photoperiod requirement. Milne apparently did not use any light while rearing the animals. Willson (1960) reared several species of Collembola under either continuous light or dark conditions and found that " only a few specimens of Sminthurus quadrimaculatus, Sinella caeca , S. curviseta and Entomobryoides purpurascens survived for any length of time, and these were in the light ". Green (1964b) reared Folsomia candida Willem using a 12 hour photophase, while Marshall and Kevan (1962 and personal communication) did most of their rearing of the same species in continuous darkness. Vail (1965) states that efforts were made to eliminate differences in light intensity in

his studies of Hypogastrura manubrialis (Tullberg). Joose (1965) mentions that Entomobrya nivalis (Linnaeus) is more active during the day and avoids such shady areas as those under the roofs of pitfall traps. The successes and failures among rearing attempts suggest that the presence or absence of light is not important in the lives of Collembola dwelling deeper in the soil than the humus layer, but light may be essential to some surface dwelling forms.

There is rather more information about the temperature requirements of Collembola. In general, Collembola may show preferences for certain temperatures but are able to endure wider temperature ranges. Agrell (1941) indicates that soil surface Collembola show a preference for temperatures above 15°C. while deep-living soil animals prefer lower temperatures.Records such as those of Hammer (1953) and abundant material in the Lyman Entomological Museum, Macdonald College, and the National Insect Collection, Ottawa, indicate the ability of some Collembola to withstand the rigorous climates of the Canadian Arctic Archipelago and alpine regions, while the experimental work of Willson (1960) shows that some Collembola can survive, at least for a short period, temperatures as high as 54.5°C. One species, Sminthurus viridis Linnaeus, is recorded by Hammer (1953) from various northern localities including Reindeer

Station, MacKenzie District, North West Territories, Canada and Greenland, while what has been taken to be the same species (and is certainly closely related) is a pest in temperate Australia (Holdaway, 1927; MacLagan, 1932). Two statements are of interest, the first by Hammer (1953): " It is a well known fact that representatives of the highly specialized families: Sminthuridae and Entomobryidae become rarer toward the north owing to their slight power of resistance and the lack of suitable vegetation types such as tall vegetation and forest, to whose bark and foliage some are attached, whereas the primitive forms which often live in the cavities of the soil are able to thrive almost everywhere.". The second quotation is after Bellinger (1954) who states: " It therefore seems likely that Collembola can endure the normal range of soil temperatures and that the latter cannot be a factor in their distribution, at least so far as the extremes of temperature are concerned.".

Rearing studies have yielded some information about the temperature relations of Collembola. Agrell's results have been referred to above. None of the Onychiurids reared by Milne (1960) developed at 24°C., but success was achieved at 5° and 12°C. Marshall and Kevan (1962) reared Folsomia candida Willem successfully at temperatures ranging from 16 to 26°C. Hale (1965b) reared deep soil onychiurids at 15°C. The work of Kyle and Long (1965) shows that Folsomides parvus Folsom and Tullbergia clavata Mills develop more

rapidly at 26°C. than at 15°C. The higher temperature is probably higher than that which would normally be encountered by this species. A survey of the literature indicates that Collembola have been reared successfully at temperatures ranging from 5° to 26°C., depending on the species. Since E. purpurascens is a surface dwelling form, a relatively high temperature is indicated. With the abundance of commercial equipment available the maintenance of suitable rearing temperatures should not present any difficulty.

Few studies have been made of the food of Collembola, the only major works being those of Macnamara (1924) and Poole (1959). The former divides the Collembola on the basis of the presence or absence of a molar plate, those having it being usually vegetarian; the others are allegedly carnivorous. He records springtails feeding on rotting vegetable matter, fungi, algae, diatoms, maple sap, pollen, other Collembola, human flesh, and a variety of commercial crops. Poole (1959) examined the gut contents of Collembola finding the following: fungal hyphae and spores, guard and phloem cells, tracheids, stone cells, mineral particles, testate amoebae, earthworm setae and collembolan scales. The work of Murphy and Doncaster (1957) and an observation by Brown (1954) show that Collembola will feed on nematodes. In general, Collembola will eat most organic matter, although it is likely that they have preferences.

The control of mold represents a major problem in the rearing of Collembola and several methods have been used for this purpose. Milne (1960) sterilized his containers before occupancy and then brushed the eggs with distilled water periodically. Marshall and Kevan (1962) used 1;1000 terpenol to control mold. "Moldex", which is commonly used in Drosophila cultures (Gardner,1961), controls molds without affecting either animals or yeasts. Vail (1965) used this compound to control mold in Collembola cultures.

Carbon dioxide is often used as an anaesthetic for temporarily quietening arthropods. It has been recorded (Dalmat, 1950) that this treatment may affect oviposition in Simuliidae but it is not known if the gas has any side-effects upon the physiology of Collembola. More recently other workers have shown some of the physiological effects of carbon dioxide on other insects (Brooks,1965; Edwards and Patton, 1967; Edwards, 1968). Vail (1965) has used carbon dioxide for temporarily immobilizing Collembola.

LIFE HISTORIES OF COLLEMBOLA

The life histories of Collembola are quite variable, differences occurring both between and within species.

Among many species of Collembola, oviposition occurs in the field only at certain regular times of the year. Hale (1965a) gives the laying dates for a number of species.

The oviposition patterns are as follows: spring only, late summer and fall only, all summer and fall, and finally spring and fall with a break in the summer. Those with a split oviposition period have either two generations or a break in oviposition caused by the low temperatures of winter. Concerning the others, Bellinger (1954) states that most species of Collembola breed continuously, the exceptions known at the time being the Tomoceridae and the Sminthuridae. The Collembola reared by Milne (1960) had life cycles in the laboratory which suggested for different species, one, two and several generations per year.

Paclt (1956) states that the eggs of Collembola are laid in batches. Hale (1965a) showed that Tomocerus minor lays its eggs singly, if possible in crevices, while Dicyrtoma fusca (Lucas) and D. minuta (O.Fabricius) deposit their eggs over the entire medium, especially in the crevices. Among the species which do lay in batches, the same preference for crevices is shown. Folsomia similis Bagnall lays its eggs in heaps (Sharma and Kevan,1963) as does Folsomia candida Willem (Marshall and Kevan, 1962). Many species lay their eggs in the holes left in the plaster medium by air bubbles (Hale,1965a).

Collembolan eggs, when first laid, are spherical. If an egg is fertile it increases in size through water intake

from the atmosphere or substrate. This expansion splits the chorion, usually into two subequal pieces which remain attached to the poles of the egg. The exposed serosal cuticle may remain smooth or may, as occurs in some entomobryids, form ridges or spines or hair-like appendages. Late in development color changes may occur, first from the eye spots and then from the rest of the embryo showing through the membranes. Incubation times have been worked out for a number of species through laboratory rearing or field studies. Hale (1965a) recorded the field incubation periods of a number of species of Hypogastrura, Onychiurus, Tullbergia, Isotoma, Isotomurus, Lepidocyrtus, Tomocerus and Dicyrtoma. All showed an inverse relationship between temperature and developmental time within the range of the habitat. Milne (1960) reared seven species in the laboratory, obtaining a similar correlation. Strebel (1932) obtained similar results with Hypogastrura purpurascens (Lubbock), showing incubation times of 28 days at 10°C. and 19 days at 22°C. (Milne's table clearly transposes Strebel's data). Thibaud (1968) reared six species in the laboratory. These all showed an inverse relationship between temperature and incubation period over the range 0° to 27.5°C. The greatest range was shown by Typhlogastrura balazuci (270 days at 0°C., 23 days at 18.5°C.). Overall the different species of Collembola may show incubation times from

6 to 270 days.

Sperm transfer in the Collembola takes place by means of spermatophores or, in a few cases, by means of direct contact of the genital plates. Spermatophores of Collembola take the general form of droplets, covered by a thin pellicle and mounted on a stalk, the whole being in the range of 250-700μ in length with a droplet diameter of 50 to 100μ . Schaller (1954) discussed spermatophores of Collembola and other primitive arthropods. Hale (1965a) has confirmed the presence of sperm in the spermatophores of Onychiurus tricampatus Gisin and O.furcifer Borner but does not mention the size of the spermatozoa. Mayer (1957) illustrates the sperm of Orchesella villosa Geoffroy and Allacma fusca(Linnaeus), giving head sizes of 1.8 to 2.4μ and 6 to 8μ respectively and tail lengths of 25 to 31μ and 50 to 60μ . Hale (1965a) has reviewed the literature on spermatophores and sperm transfer.

Parthenogenesis is known to occur in some species of Collembola and is suspected in others. The first suspicion of parthenogenesis was recorded by Nicolet (1842). Falkenhan (1932) showed experimentally that parthenogenesis does not occur in Sminthurides aquaticus (Bourlet). Schaller (1953) reared in isolation Onychiurus armatus Protaphorura armata (Tullberg) , Orchesella cincta (Linnaeus) , O. villosa (Geoffroy), O. flavescens (Bourlet), Tomocerus

vulgaris (Tullberg), T. longicornis (Muller), and Dicyrtoma (Dicyrtomina)minuta (Linnaeus). Of these O. armatus and the tomocerids laid no eggs. Five out of the 478 Orchesella laid eggs, none of which were viable. About ten per cent. of the Dicyrtoma laid eggs, none of which developed. Mayer (1957) states that his observations suggest that parthenogenesis does not occur in Collembola. Choudhuri (1958) demonstrated parthenogenesis in Onychiurus parthenogeneticus Choudhuri. Similarly, Marshall and Kevan (1962) and Green (1964a,b) have demonstrated parthenogenesis in Folsomia candida (Willem). It appears that only a small proportion of Collembolan species reproduce parthenogenetically.

After hatching, a collembolan undergoes 4 to 12 molts before it reaches maturity (Lindenmann, 1950; Milne,1960; Marshall and Kevan, 1962). In many cases the differences between the instars are very slight, making the separation of the instars difficult. Separations have been made by measurements of the anal aperture (Betsch, 1967) and the length of the head capsule (Hale, 1965b; Milne, 1960). Thibaud (1967b) studied a number of measurements: the lengths of: unguis III, unguifer III, tibiotarsus and pretarsus III, mucro, dens, anal spine, postantennal organ, head, thorax and abdomen and the diameter of the second cornicle. He showed the relationships between these dimensions and age, based on actual rearing data.

Agrell (1948), working solely on preserved material reached various conclusions concerning number of instars, growth increments, and applicability of Dyar's and Przibram's laws to several species of Collembola. Similarly, Janetschek (1967) formed conclusions from a massive statistical analysis of the distribution of overall lengths of preserved specimens of Gomphiocephalus hodgsoni Carpenter. While the conclusions of the last two workers may in fact be valid, they cannot be fully accepted in the absence of supporting rearing data. Changes in the arrangement and number of the setae have been used with some success by Thibaud (1967b) working on Hypogastruridae and Hale (1965b) on Onychiuridae. Lindenmann (1950) was able to separate the stages of some Swiss species of Orchesella through changes in color patterns. Uchida and Chiba (1959) used measurements of antennal segments, body length and their ratios in the separation of instars of Tomocerus minutus Tullberg. Christiansen (1954) studied the use of measurements and ratios of measurements in Entomobrya and Entomobryoides and concluded that the intraspecific variation was often as great as the interspecific variation. This would preclude the use of measurements for the separation of instars.

The sex ratios of Collembola vary greatly between species and probably within species. Hale (1964) found no males in any except one of a number of populations of

Onychiurus procampatus Gisin, the exception showing 32% males. In the same study, O. tricampatus showed a range of 18 to 47% males at different localities on the same day. Thibaud (1967b) recorded sex ratios of populations of Hypogastruridae, ranging from 40 per cent. males for Schaefferia willemi (Bonet) to 60 per cent. males for Mesachorutes quadriocellatus Absolon.

DIAPAUSE

A number of definitions have been proposed over the past few years, none of them fully satisfactory. The term was originally proposed by Wheeler (1893) to describe a stage in the embryogenesis of a Tettigonioid orthopteran, Xiphidium ensiferum Scudder [= Conocephalus ensifer (Scudder)]. The present usage dates from the change made by Henneguy (1904) who applied the name to a condition of arrested growth.

Simmonds (1948) states that diapause is a state " in which a reduction of growth processes or maturation occurs which is not necessarily caused by immediate environmental influences, does not depend for its continuation on unsuitable conditions, and is not easily or quickly altered by change to a more favorable environment. However, once the state of diapause comes to an end, normal growth and development are resumed.".

Andrewartha (1952) states that diapause may be considered as a stage in physiogenesis which must be completed as a prerequisite for the resumption of morphogenesis.

Beck (1968) states " Diapause is a genetically determined state of suppressed development, the manifestation of which may be induced by environmental factors.".

Lees (1955) has summarized most of the earlier knowledge concerning diapause.

As the changes associated with diapause vary among species, so the criteria used to identify the state vary. Initially, the task is to establish that there is, in fact, a state of suppressed development. Vinogradova (1965) has described clearly the manner in which she demonstrated such an interruption in the life cycle of some specimens of the mosquito, Aedes togoi Theobald. A few animals show differences in external morphology between the diapause and active states, but many have only internal differences. Brazzel and Newsom (1959), for example, have made use of the differences in the testes, ovaries, fat, moisture content and respiratory rate in their study of diapause in the weevil, Anthonomus grandis Boheman. There are many other physiological changes in diapausing insects, but few of them are useful for the demonstration of the existence of a diapause, so they will not be discussed here.

III. MATERIALS AND METHODS

COLLECTION

All of the animals used in this project were collected in the Morgan Arboretum, Macdonald College, Ste. Anne de Bellevue, Province of Quebec, or were the progeny of animals collected in that locality. While Entomobryoides purpurascens may be collected from under dead bark, in this present study all collections were made from a wood pile, from which large numbers were readily available. The wood in this pile consisted of a mixture of tree species, mostly beech (Fagus) and birch (Betula).

Collections were made with a simple aspirator. All stages except the first two or three instars could be collected easily by turning over logs and removing the disturbed residents. The aspirator deposited the animals in a plastic snap-cap vial * with a base of plaster of paris and charcoal. The animals could then be kept in these vials until they were needed in the laboratory. The vials could not be used for rearing because the type of plastic was found to inhibit totally the hatching of eggs of this species (see Table I), although the active stages of the animal did not appear to be affected, at least on a short-term basis.

* More information about pieces of equipment and materials marked with an asterisk is provided at the end of this section.

REARING

EQUIPMENT

Experience with other species of Collembola has shown that a high humidity is usually required. The results of earlier trials in the present project indicated the need for a controlled photoperiod. The strict control of temperature was dictated by the nature of the experiments rather than the needs of the animals. In addition most of the experiments demanded ease of observation. The equipment described below was designed to satisfy all of these requirements.

Temperature was maintained in incubators * (above room temperature) and a household type refrigerator * (below room temperature). The calibrations of the incubator thermostats were standardized against laboratory mercury thermometers. Temperature variation in the incubators was $\pm$ 1°C. while in the refrigerator it approached $\pm$ 2°C.

Illumination was provided by a 10 w. incandescent light bulb * controlled by a time switch *. Intensity of illumination at the level of the rearing chambers varied between 15 and 25 foot-candles.

Suitable humidity was maintained by means of a special rearing apparatus, based on a design originally conceived by Mr. Lynton Martin of Sault Ste. Marie, Ontario, and subsequently much modified by various graduate students

of the soil zoology laboratory of the Department of Entomology, Macdonald College. Each rearing container consisted of a clear plastic box * divided into two chambers, one measuring 11 x 3½ and the other 11 x 10½ inches (28 x 9 and 28 x 27 cm.). The floor of the larger chamber was covered to a depth of about a quarter of an inch (0.6cm.) with a plaster of paris / charcoal mixture. Circular holes 1½ inches in diameter (3.7 cm.) were made in the sides of both chambers, one connecting them and the other providing a vent to the exterior. The smaller chamber was provided with an evaporating surface consisting of cotton gauze curtains hung on 12-guage iron wire. These curtains were spaced 3/4 inch (1.9 cm.) apart. Air was moved through the system by an exhaust fan powered by a small clock motor and mounted at the exit hole of the larger chamber.

Within the larger chamber the animals were confined in a variety of containers. Most of the work (involving small numbers of individuals) was done using 2- dram (11 cc. approx.) snap-cap vials * . A quarter-inch (0.6 cm.) layer of the charcoal / plaster of paris mixture at the bottom of the vials was in contact with the plaster base of the rearing chamber through holes blown in the bottoms of the vials. For rearing the larger stages of the animals the centers of the snap-caps were cut out and replaced with nylon cloth. When the younger stages were reared, solid caps were used to minimize the risk of the animals escaping.

For the rearing of larger numbers, plastic boxes *, measuring 3½ by 2 3/4 by 1½ inches (9 x 7 x 3.5 cm.) were used. After two years of use, however, it became evident that the plastic of these boxes inhibited, or at least retarded, the growth of active E. purpurascens. For this reason, the use of these boxes was discontinued. It should be noted that there are many types of plastics and that these vary in the degree to which they inhibit biological processes. This probably accounts for the differences in effect between the plastics of the vials (described earlier) and of the boxes.

In rearing where frequent observation was not necessary, a different type of container was used. This was a screw-cap jar with a base consisting of an inch of moist sand covered with a base of plaster of paris / charcoal. Such jars kept maintenance to a minimum, but could not easily be searched for the presence of eggs or small young. This type of container has often been used by other workers for mass rearing. Light and temperature conditions were maintained in the same manner as with the other containers.

DEVELOPMENT OF REARING TECHNIQUE

Rearing attempts were made under the nine sets of conditions listed below. Except where other conditions are indicated, all rearing trials employed the small

2-dram glass vials described earlier, with 6 to 10 animals per vial. All animals were fed on yeasts and volunteer molds.

1) Animals were collected at various dates between May and October and reared in darkness at 18°C.

2) Animals collected at various dates between May and October and reared with $15^{3}/4$ hour photophase at 18°C. These conditions were chosen empirically as being what appeared to be optimum conditions in preliminary tests.

3) Animals collected at various dates between May and October. Reared in plastic boxes at 18°C. with a $15^{3}/4$ hour photophase.

4) Animals collected October 15. Stored at 10°C. approx. in a household refrigerator until December 5, then moved to 18°C and a $15^{3}/4$ hour photophase.

5) Animals collected from May to July and reared at 25°C and a $15^{3}/4$ hour photophase.

6) Animals collected from May to July and reared at 25°C. and a 16 hour photophase.

7) Animals collected in September and reared at 25°C. and a 16 hour photophase.

8) Animals collected in September, stored at 5°C and a 10 hour day for one or two months and then moved to 25°C. and 16 hours photophase.

9) Animals collected in July, reared at 18°C. and $15^{3}/4$ hour photophase in plastic snap-cap vials.

DEMONSTRATION OF DIAPAUSE

One hundred-and-seventy E. purpurascens were collected from the Morgan Arboretum on September 25, 1968. They were divided into lots of ten and reared in the two-dram vials on the usual yeast and volunteer molds. Groups were set up on different rearing regimes as follows:

1) Control: eight vials of animals were kept at 25°C. and a 16 hour photophase and kept under observation until the last animals dided .

2) Five vials were kept at 5°C. and 10 hour photophase for one month and then transferred to 25°C. and 16 hours.

3) Five vials were kept at 5°C. and 10 hour photophase for two months and then transferred to 25°C. and 16 hours.

Survival was recorded at the ends of the first two months.

MOLD CONTROL AND ANAESTHETICS

At times it was necessary to use some form of mold control in the cultures. All vials were rinsed with 70 per cent. ethanol and allowed to dry before use. This treatment alone was sufficient for short term rearing. In longer term rearing, other than experimental rearing, "Moldex"[1] (see Gardner, 1961) was used as a control agent. While no ill effects on the Collembola were observed, the

1. Methyl p-hydroxybenzoate

use of the agent in experimental series was avoided because of the slight possibility of interference with the results. Terpenol, which has been used in the soil zoology laboratory at Macdonald College, was not used because of suspected (but as yet unconfirmed) undesirable effects on the animals.

Carbon dioxide was used as an anaesthetic when transferring animals. While the use of any anaesthetic is undesirable because of possible effects on physiology and development, in this work, due to the active habits of the animals, the use of the gas was unavoidable.

STUDIES OF PRESERVED MATERIAL

MEASUREMENTS

Measurements have been used as a basis for the determination of the number of stadia in the life of some species of Collembola and other arthropods. The measurements described below were made with a view to assessing the applicability of this technique to E. purpurascens.

All specimens used were killed in 70 per cent. ethanol and mounted on glass slides in Hoyer's medium covered with glass cover slips and sealed with Gurr's "Glyceel"*. Measurements were made with a Reichert "Biozet" microscope equipped with phase contrast optics and a calibrated ocular micrometer.

The structures chosen for measurement were those considered least likely to be distorted by the mounting process. Head capsule width, used in Dyar's formula, had to be eliminated on these grounds. The structures finally chosen were: antennal segments II, III, and IV; femora and tibiotarsi I and III, unguis III and unguifer III. Organs, in particular antennal segments, which were distorted through regeneration, were not included in the results. Many measurements of unguis and unguifer had to be discarded because the angle at which the structures lay precluded accurate measurement.

Two lots of specimens were examined. The first consisted of three collections from the field made on 17.XII.66, 20.IV.67 and 15.VIII.67. The second lot followed because of the lack of convincing size classes in the first. Animals of known instar, reared at 18°C. or 25°C. were measured and compared. Some animals were reared for longer periods (three months) to provide a comparison with those collected from the field.

Another series of measurements was undertaken with the object of finding the minimum size of field males on maturity. Accordingly, the third femora of all males collected in May and June, 1968, were measured.

PROPORTION OF MALES AND SEXUAL MATURITY

The first series of animals measured, those from 1966 and 1967 (see preceding section), showed that there were marked fluctuations in the proportion of mature males in the field population. In the following years (1968-69), a sampling program was undertaken with the object of showing the details of the spring changes in the proportion of mature males. Rough estimates were obtained for the fall but a conflicting schedule did not permit a complete sampling program at this time.

Because of the difficulties in identifying females, as outlined in the discussion, attention was directed to the proportion of mature males in the population rather than a true sex ratio. Mature males were identified by the presence of the characteristic male genital plate. This structure and usually the lower end of the gonoduct could be seen, regardless of the position of the furcula. While size is not a good indicator of the precise stage of development the rearing studies indicated that it was highly unlikely that an animal with a third femur length less than 210μ would be mature, so specimens below this size were not included in the results.

The material examined came from collections made in the preliminary studies of 1966 and 1967 and the more regular sampling in 1968 and 1969. The exact dates are recorded with the results.

GUT CONTENTS

The Hoyer's mounting medium clears the bodies of E. purpurascens so that the gut contents may be seen. This provides a convenient means of investigation of the normal diet of the species.

All of the animals mounted for the sex-proportion studies were further examined for the presence of identifiable gut contents. These were classified in broad categories. It was not deemed practical to attempt to identify the pollen and fungal material more precisely.

FIELD OBSERVATIONS

Limited studies were made of the activities of E. purpurascens in the field. Before any collection and associated disturbance was made, ten minutes were spent observing the activities of those animals which were on the surface of the pile. No optical aids were used.

During collection, any other species of Collembola were collected and subsequently identified and the presence of other arthropods was noted. Spot checks were made to compare air and wood pile temperatures. These temperatures were measured by means of a telethermometer* equipped with air and soil temperature probes. The soil temperature probes* were inserted into crannies in the pile, while the air temperatures were measured in the shade within two metres of the pile and at the same height from the ground as the other probe.

SPECIFICATIONS AND SOURCES OF SUPPLY

Boxes, plastic

Althor Products,

Brooklyn, N.Y.

Hoyer's Medium

50 g. distilled water

30 g. gum arabic

200 gm. chloral hydrate

20 gm. glycerine

Glyceel

G.T.Gurr, Ltd.,

London, SW6,

England.

Incubators and Refrigerator

a)Precision Scientific Co.,

Chicago, Illinois.

Model 805

b)Modern Laboratory Equipment,

New York, N.Y.

Model 560

c)Westinghouse

Model LR860

Light bulbs

Sylvania 10w., 115v.

Telethermometer and Probes

Yellow Springs Instrument Company Ltd.,

Yellow Springs, Ohio.

Telethermometer Model 44TE

Air temperature probe 405

Soil temperature probe 418

Timers

Paragon Electric Co.,

Two Rivers, Wis.

Model APT5-O

Vials, glass

Johnsen and Jorgensen, Ltd.,

London, EC4.

2-dram vials, series COC.

Vials, plastic

Jones Box and Label Co.,

London, Ontario

#7 snap-cap R_x vials

RESULTS

REARING

The development of the rearing technique is , of necessity, closely linked with the diapause study. The results of the different rearing regimes are summarized in Table I, while those of the diapause study are shown in Table II. The information on the general biology of E. purpurascens was obtained during the rearing trials or from other sources as indicated.

Oviposition occurred within a few days (see Fig. 12) and sometimes within a few hours of collection if the animals were collected in the period mid-May to early September. The date of initiation of oviposition in the field varied from May 9 (1968) to May 20 (1969), based on weekly samples from early April to late May. The oviposition lag for the early spring of 1968, when the animals were reared at 25°C. after collection, showed a steady decrease until early May, after which time it fluctuated about a low mean until the end of August, when there was a very marked increase (Figure 12).

In the laboratory environment provided, E. purpurascens usually laid eggs on the plaster of paris base. If the surface of the plaster was pitted, the eggs were usually laid in the pits, if there was room (cf. Hale 1965a). The eggs were placed singly and never in distinct heaps

as in some other species. The eggs, when first laid, were white, smooth spheres averaging 200μ in diameter. If they were infertile, no change took place and the eggs were usually eaten within a few days if active forms were left in the vials. Fertile eggs swelled within 24 hours to a diameter of about 250μ . The chorion split and the two halves remained attached to the opposite poles. Between the wrinkled hemispheres of the chorion, there was a regular pattern of ridges in the serosal cuticle (see Fig. 13). These ridges gradually smoothed out and were usually not visible on the last day before hatching. Color changes also occurred, the eggs acquiring a reddish-purple tint after a few days (4 days at 25°C.). Most, if not all, of this change was the result of first the eye spots and then the rest of the embryo showing through the membranes of the egg. The incubation periods were:

16 days at 18°C.
7 days at 22°C.
6 days at 25°C.

The durations of the first three instars are shown in Tables III and IV, while the measurements of the instars are shown in Tables V and VI.

Color changes accompanied growth. The first instar larvae were uniformly gray except for the darker eye patches, and had very few setae. During the succeeding instars the

the setae increased in number, giving the animals a brown color. At the same time the purple pigment in the cuticle increased, but this was not often noticeable in the living specimens (although it showed in the slide preparations).

The number of instars between hatching and maturity appeared to be quite variable. At the only temperature at which a life cycle was completed in the laboratory, 25°C., maturity occurred in the fifth to seventh instars. However, there is good reason to believe that this does not represent the situation in the field (see discussion).

During the course of rearing, specimens of E. purpurascens were seen feeding on yeast, molds, exuviae and the bodies of dead Collembola (cf. studies of preserved material). There was a striking variation in the appearance of the fecal pellets. The usual kind were oval, approximately 80 x 40μ and transparent in color. The other type was much less common and about three times as long as the first and orange to brown in color. Fecal pellets of the second type, because they were at one time suspected of being spermatophores, were subjected to further examination. They were squashed and examined under the light microscope, which revealed a thin-walled sac filled with very active bodies about 0.75μ in length. Subsequent electron microscope studies showed that the bodies were flagellate bacteria.

STUDIES OF PRESERVED MATERIAL

MEASUREMENTS

The distribution of size classes among the measurements of animals collected from the field are shown in the histograms in Figs. 1 to 9. The results are divided into three groups, numbered 250-310, 311-438 and 458-497, which represent collections made on 17.XII.66, 20.IV.67 and 15.VIII.67, respectively.

The measurements of specimens reared in the laboratory are shown in Tables V and VI.

The measurements of mature males collected in the field are shown in table VII and those of animals reared in the laboratory for three months are in table VIII.

SEX PROPORTION STUDIES

The percentages of mature males in the samples are shown in Tables XII, XIII, and XIV. They are summarized in Figures 10 and 11.

GUT CONTENTS

The results of the study of gut contents are shown in Tables IX, X and XI. Fungal spores and hyphae accounted for most of the identifiable gut contents, while pollen and collembolan remains were less frequent. The table is largely self explanatory but it should further be stated that there was no material (with the exception of pollen grains) which could positively be identified as higher plant tissue. There were several fragments which were suspected of being

leaf or stem tissue, but these were in poor condition and could not be confirmed. There may also have been a few algal cells mixed in with the fungous matter but, in most cases, it was possible to rule out this possibility; algae were not prominent in the habitat.

A number of specimens contained reddish-colored bodies in their guts. These were apparently food masses encased in a peritrophic membrane. They appeared to be identical with the larger type of fecal pellet described earlier. Their frequency is indicated in Tables VXI to IXI.

FIELD STUDIES

E. purpurascens, when living in woodpiles, was found in the more sheltered angles of the logs and among the entrapped leaves or around fungus growths on the bark. As the logs decayed and the bark weparated from the xylem, increasing numbers of the animals were found between these tissue layers. During the summer a number of the animals (but probably representing only a small percentage of the woodpile population) were seen roaming about on the exposed surfaces of the wood pile. The activity was observed only in the morning, never in the afternoon, and its extent appeared to bear no noticeable relationship to the amount of direct sunlight, for the animals ran indiscriminately in and out of patches of sunlight (in the laboratory, too, the animals were not disturbed by microscope lights).

In the winter the collembolans stayed in their refuges, but were still active in temperatures as low as -2°C.

Several other species of Collembola were present in the same woodpile. None of them could easily be confused with E. purpurascens, even without optical aids. These other species included Entomobrya clitellaria Guthrie, E. nivalis (Linnaeus), E. marginata (Tullberg)?, E. quadrilineata Buecker, Lepidocyrtus cyaneus Tullberg, L. paradoxus Uzel, L. violaceus Geoffroy?, Tomocerus sp., Isotoma viridis Bourlet, I. olivacea Tullberg, Hypogastrura sp., and some unidentified Onychiuridae and Sminthuridae.

In addition to the Collembola, there were present the usual wood pile fauna of mites, spiders, centipedes, millipedes, ants, sawflies, beetles (including a number of Staphylinidae), thrips, moths (and caterpillars) and psocids.

The results of the spot checks of temperature are shown in Table XV.

IV. DISCUSSION

The previous section set out a number of rather isolated studies, all designed to clarify some aspect of the biology of Entomobryoides purpurascens. Some of the routes followed in the search for information are rather circuitous because the obvious ways which might be feasible in an insect, for example, are closed by quirks of collembolan biology. It would perhaps be instructive at this point to examine some of these difficulties.

First, Collembola are small: most species are less than one millimetre in length, although E. purpurascens may grow to 3 mm.. Collembola continue to molt after maturity and, it is reported, may molt as many as fifty times during their lives (Paclt, 1956). In most cases there is no obvious morphological difference between mature and immature individuals. There are few secondary sexual characteristics and it is usually impossible to sex living animals. The only character that is at all reliable for the sexing of Entomobryidae is the genital plate of the male. This ring of setae surrounds the genital opening and can easily be seen with the higher powers of the compound microscope, providing a positive identification of a dead male, but can rarely be seen in a living specimen. The female can rarely be identified with certainty, for its genital plate is simple, like that

of immature forms. The genital openings of both sexes are located under the abdomen immediately posterior to the furcula. Thus the structures are obscured and distorted when the furcula is extended and it is often impossible to distinguish between mature females and immature specimens of either sex. The location of the genital structures makes it impossible to sex the living animals, especially in situations where anaesthesia is undesirable.

Parthenogenesis is rare among Collembola and probably never occurs in E. purpurascens. Since the sexes cannot be identified, numbers for rearing in a single container have to be chosen with a view to sufficient probability of the presence of a male. If eggs are laid in a culture there is no evidence by which one can tell which, or how many, of the adults are ovipositing. The frequent molts preclude any effective marking of individuals, as does their size.

If the above obstacles are not enough, the retiring habits of E. purpurascens present more difficulties. The animals live in secluded crannies or under bark. The rolling over of a log to expose the animals constitutes a major disruption of the environment, one which may not be rectified for days, or even weeks. The shelters are fairly effectively sealed with leaves and other debris. The insertion of probes for the measurement of humidity would probably cause sufficient disturbance to render any readings thus

obtained valueless. Direct observation of most aspects of the biology of the animal in the field are virtually impossible.

The preceding paragraphs explain the difficulties. Let us now examine the information which was obtained. As a starting point, early April will suffice.

In early April, the animals are active and feeding (Tables IX, X and XI). There are no mature males in the population (Tables XII, XIII,XIV, Figs. 10, 11), but it is possible that some females are mature at this time. Little change takes place until the end of April or early May. Then, at a date dependent on the severity of the preceding winter, the first males appear. Within a week the proportion of males reaches its summer level of 17 to 30 per cent. By the following week the females have apparently been fertilized and are ready to lay eggs. At this time they oviposit immediately on being brought into the laboratory. Oviposition is initiated in the second half of May. Even then, the temperatures in the habitat are low, for wood is a good insulator and, in addition, the study area was shaded. On the basis of the laboratory rearing times the incubation period would be well in excess of 16 days, perhaps over 40. This indicates that the first young hatch in late June or early July. The spot checks indicated that in July and August the wood pile temperatures reached 20°C. and occasionally 25°C. during the day. The night temperatures were

not recorded but a drop of 5°C. is not unlikely. This indicates a daily mean of 15 to 20°C. and a duration of approximately seven to nine days per stadium. On a basis of seven instars to reach maturity, the young could not attain this state before mid-August. Further, the differences between the measurements of field-collected males and laboratory reared animals indicate that there are probably more than seven instars before maturity under natural conditions. There is, nevertheless, a slight possibility that in a hot summer following a mild winter there is a second brood at this time. In most years, however, it is unlikely that this takes place for, despite warm temperatures which may continue into October, the animals go into diapause around the first of September. Oviposition ceases and mature males disappear from the population. Only a small proportion of the animals continue to feed (Tables IX and X). Collections brought into the laboratory and kept at 25°C., normally a good rearing temperature, died without ovipositing. The collembolans pass the winter in their refuges and by late January, the physiological requirements for the termination of diapause are at least partially completed. The delay between collection and oviposition, about 40 days in late January, steadily decreases at a rate of change probably dependent on the severity of the winter (Fig. 12). This completes the life cycle.

Before considering some of the side issues arising

from the life-cycle study, the feeding habits of E. purpurascens remain to be examined. Fungal matter makes up the bulk of the normal diet. The only other important constituent is pollen. This is apparently wind-borne, coming from two sources, the surrounding trees in the spring and a different source, perhaps ragweed or goldenrod, in the summer. The other consistent contents of the gut were collembolan remains. Most of these were probably of E. purpurascens, but in one case they were those of another species, Lepidocyrtus sp. The animals are probably only scavengers; no evidence of predation has been seen in the laboratory. They have been seen eating exuviae and the bodies of dead Collembola. Of interest is the fact that no higher plant material other than pollen was found in the guts. Further, the possibility is remote that any significant amount of the unidentified material was of this origin. This suggests that E. purpurascens does no direct damage to wood or leaves although it may cause indirect damage by spreading spores.

We may now examine a few isolated points, starting with the eggs and their development. The swelling of the eggs and associated splitting of the chorion and development of ridges is similar to that of other related groups. There was no evidence of finger-like projections as was reported (Uchida and Abukawa, 1956) for Tomocerus minutus Tullberg and other species. Development times were recorded at three different temperatures. There was a slight increase

between 25° and 22°C. and a very marked jump between 22° and 18°C. Projection of this trend leads to the possibility of extremely slow development at lower temperatures, such as are found in the field in May and June.

The determination of the number of instars to maturity presents problems. In the past, as mentionned in the literature review, workers have attempted this with measurements of large samples and subsequent elaborate statistical analysis. Their conclusions would have been more convincing, however, had they been supported by rearing data. The three series which were measured in this project yielded no good size classes (Figs. 1 to 9). It was expected that the first two or three instars would be missing, but, in theory, there should still have been four or five distinct peaks in the frequency distribution of some of the measurements. Consequently the rearing studies at 18° and 25°C. were undertaken. The results were illuminating. The two series had markedly different size classes. Furthermore, within the temperature series, there were overlaps of measurements of structures between instars. The measurements of some instars at 25°C. were almost identical with those of other instars at 18°C. One can conclude from this that, in the variable temperatures of the natural habitat, the instars would be hopelessly mixed and that no statistical analysis of measurements would yield meaningful results. It is also worth noting that the series at the lower temp-

-erature were smaller than the 25°C. series, pointing to much smaller animals at field temperatures.

Diapause has proven to be an important factor in the life of E. purpurascens. To date, circumstances have permitted only the demonstration of the existence of a diapause and a study of the breaking of diapause. Initially it was known that oviposition ceased in late August or early September despite the fact that warm temperatures continued until later in the fall. Throughout the inactive season there were always animals present which were large to be mature but which showed no adult sexual characters. It was also known that in the laboratory at 25°C. the animals would complete a life cycle in 30 to 35 days.

The existence of a diapause was tested as outlined on page 25 , with a control at 25°C. and the other series with different cold treatments intended to satisfy the requirements of diapause development. With one exception, no eggs were laid in the control cultures. The single case of oviposition can be attributed to normal variation within a species. Mortality in the control culture was high when compared with the experimental series (Table II). Eggs were laid in 40 per cent. of the cold treated cultures. In some of these cultures large numbers (100+) of eggs were laid. These developed satisfactorily (Table II). In all cases, these animals, which had been large enough to be mature at the start of the experiment, still required a

minimum of 20 days at 25°C. before oviposition plus the period at 5°C. This shows that an arrest definitely takes place and that the requirements for diapause development can be fulfilled by between one and two months at 5°C.

The cause of the arrest is not yet clear. It is clear, however, that there is no obligate diapause because it is possible to rear the animals without diapause in the laboratory. Photophase is suspected as a cause of arrestation because there appears to be no other consistent environmental change at the time the animals enter diapause. There is a possibility that lower night temperatures also play a part, but both of these hypotheses have yet to be tested.

There appear to be two classes of animals entering diapause in the fall. One is the normal new generation. The other consists of animals that have already lived through one reproductive season, for there were individuals in the population in the fall which were much too large to have belonged to the brood of the same year, exceeding the size of third instar juveniles reared at 18°C. by a factor of three or four. The size of these animals was comparable with that of animals which had undergone nine or more molts in the laboratory (Table VIII).

It seems that these large animals may go through a second reproductive season, losing their external sexual characters in the interval. This is not as improbable as

it might seem, for a similar situation has been shown by Verhoeff (1933, 1939) to occur in some millipedes, the hexapod larval stages of which exhibit certain features reminiscent of Collembola. If a method can be developed for reliably sexing living adults, then animals could be reared in pairs so that this hypothesis could be tested and the problem of the numbers of eggs laid by individual females could be solved.

None of the other species of Collembola listed as being present in the habitat is abundant, and none of them is easily confused with E. purpurascens. It is of interest to note, however, that the fauna includes Lepidocyrtus paradoxus Uzel, a European introduction (Snider and Fischer, 1964) which was not recorded in previous surveys in the Morgan Arboretum (Marshall, 1963, 1967). Of the other arthropods present in association with E. purpurascens, the Staphylinidae and Micryphantidae may be predators of that species.

V. SUMMARY AND CONCLUSIONS

1) Requirements are outlined for the laboratory rearing of E. purpurascens. In addition to the usual conditions demanded by Collembola, this species has a light requirement.

2) The time of onset of oviposition in the field varies from mid- to late May, apparently depending on the severity of the preceding winter.

3) Oviposition continues throughout the summer until approximately September 1.

4) On approximately September 1, the active stages of E. purpurascens enter a state of diapause which can be broken by a period of chilling (1 to 2 months at 5°C.).

5) E. purpurascens normally has a one-year cycle in the field but some animals may live two years or more. This may possibly involve the loss of sexual characters in the interval between seasons.

6) The proportion of mature males among animals of sufficient size to be mature fluctuates between 17 and 30% from early May until late August or early September. For the remainder of the year mature males are absent from the population.

7) The natural diet of E. purpurascens consists of fungal spores and mycelium, pollen and collembolan remains.

8) Rearing temperatures have a marked influence on the sizes of the first three instars of E. purpurascens.

9) Measurements are of no value in the separation of the stages of E. purpurascens.

10. Plastics and mold-inhibiting chemicals may retard or totally inhibit growth in this species under laboratory conditions.

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TABLE I

A comparison of different rearing techniques. The treatments are explained in detail on page 24 .

Treatment	Collection date	Oviposition	Progeny Hatched	Progeny Matured	Progeny Oviposited
1	May-Aug	+	+	-	-
	Oct.	-	-	-	-
2	May-Aug.	+	+	-	-
3	May-Aug.	+	+	-	-
	Oct.	-	-	-	-
4	Oct.	+	+	-	-
5	May-Aug.	+	+	+	+
6	May-Aug.	+	+	+	+
7	Sept.	*	-	-	-
8	Sept.	+	+	+	+
9	July	+	-	-	-

+ indicates that an activity took place

- indicates that an activity did not take place

* one isolated case of oviposition

TABLE II

Rearing results and survival records for groups of Entomobryoides purpurascens used to demonstrate the existence of a diapause in the species.

Vial	Treatment	Survival			Oviposition	Mature offspring
		25.IX	25.X.	23.XI		
802	1	10	4	0	-	-
803	1	10	5	0	-	-
804	1	10	0	0	-	-
805	1	10	4	0	-	-
806	1	10	2	0	-	-
807	1	10	5	3	21.X.68	-
808	1	10	2	0	-	-
809	1	10	6	0	-	-
810	2	10	9	5	17.XII.68	+
811	2	10	8	4	-	-
812	2	10	9	3	9.XII.68	+
813	2	10	10	3	-	-
814	2	10	10	5	-	-
815	3	10	*	7	-	-
816	3	10		5	12.XII.68	+
817	3	10		8	-	-
818	3	10		7	17.XII.68	-
819	3	10		8	-	-

* Animals could not be removed from cold for counting without invalidating other results.

TABLE III

Duration of stages of Entomobryoides purpurascens reared in the laboratory at 18°C., based on observation every 24 hours.

Instar	Duration in Days	Replicates
Egg	16	42
1st.	8.2	42
2nd.	7.2	38
3rd.	6.4	22

TABLE IV

Duration of stages of Entomobryoides purpurascens reared in the laboratory at 25°C., based on observation every 24 hours.

Instar	Duration in Days	Replicates
Egg	6.3	17
1st.	2.9	62
2nd.	2.7	52
3rd.	2.9	28
4th.	2.8	19

TABLE V

Ranges and average measurements of lengths of indicated structures from samples of Entomobryoides purpurascens reared in the laboratory at 18°C. Averages in parentheses. All measurements in microns.

Organ	1st. instar	2nd. instar	3rd.instar
Antenna II	39-43	53-59	59-70
	(42.5)	(55.8)	(66.2)
Antenna III	43-47	53-61	55-74
	(44.6)	(57.0)	(67.3)
Antenna IV	82-94	102-117	117-131
	(89.3)	(112.0)	(125.7)
Unguis III	16-18	18-23	23-27
	(17.1)	(19.6)	(23.7)
Unguifer III	8.12	10-14	14-16
	(11.3)	(12.0)	(14.3)
Femur I	55-61	68-80	78-121
	(57.2)	(74.2)	(91.7)
Tibio-	70-78	82-92	94-131
tarsus I	(75.1)	(90.5)	(107.2)
Femur III	68-76	90-98	107-123
	(74.2)	(93.5)	(119.8)
Tibio-	86-111	123-139	148-166
tarsus III	(104.0)	(132.1)	(155.4)

TABLE VI

Ranges and average measurements of lengths of indicated structures from samples of Entomobryoides purpurascens reared in the laboratory at 25°C. Averages in parentheses. All measurements in microns.

Organ	1st. instar	2nd.instar	3rd. instar
Antenna II	48-53	62-70	95-127
	(49.7)	(65.8)	(105.5)
Antenna III	43-58	62-72	81-120
	(52.3)	(65.3)	(102.5)
Antenna IV	82-110	120-139	158-197
	(104.4)	(126.2)	(180.0)
Unguis III	17-24	22-24	26-31
	(21.5)	(22.0)	(28.8)
Unguifer III	12-14	12-14	14-22
	(13.2)	(13.4)	(17.5)
Femur I	67-74	72-86	118-158
	(69.2)	(84.1)	(126.5)
Tibio-	86-96	96-108	132-178
tarsus I	(90.4)	(102.5)	(157.7)
Femur III	84-91	108-115	144-187
	(88.5)	(112.9)	(164.6)
Tibio-	108-132	151-159	192-260
tarsus III	(127.6)	(154.0)	(234.7)

TABLE VII

Lengths of third femora of male Entomobryoides purpurascens collected from the Morgan Arboretum, Macdonald College, on indicated dates.

Date of Sample	Average Length in microns	Standard Deviation in microns	Replicates
6.V.68	387	42	12
13.V.68	384	11	11
27.V.68	359	26	12
11.VI.68	372	39	9
25.VI.68	439	45	16
15.VIII.67	353	38	8

TABLE VIII

Lengths of third femora of Entomobryoides purpurascens reared in an incubator at 18°C. from 13.IX.67 to 23.XII.67 (Rearing begun in first instar)

Animal Code No.	No. of known molts	Length in microns
246	12	273
249	10	318
251	12	364
253	9	337

TABLE IX

Gut contents of samples of Entomobryoides purpurascens collected in the Morgan Arboretum, Macdonald College, in 1966-1967

Collection date	No. in sample	No. of specimens containing: Indet. debris	Pollen	Spores	Hyphae	Collembolan remains *
17.XII.66	60	58	-	-	-	2
20.IV.67	128	100	-	18	20	1
15.VIII.67	38	19	-	15	13	1

* Because the contents of the guts were often mixed, the totals for all food categories may exceed the number of animals in the sample.

TABLE X

Gut contents of samples of Entomobryoides purpurascens collected in the Morgan Arboretum, Macdonald College in 1968

Collection date	No. in sample	Indet. debris	No. of specimens containing: Pollen	Spores	Hyphae	Collemb. remains	* PM
15.IV.68	50	42	2	1	-	1	6
22.IV.68	50	34	7	1	5	1	-
29.IV.68	50	26	1	18	7	2	1
6.V.68	57	43	-	-	-	5	11
13.V.68	54	44	-	5	3	1	4
27.V.68	68	47	2	6	1	2	9
11.VI.68	44	17	4	24	11	2	2
25.VI.68	55	18	2	32	27	3	-
8.VII.68	55	19	2	31	17	3	-
8.IX.68	57	50	-	6	1	3	-
23.IX.68	55	43	-	5	1	1	4
30.IX.68	39	31	-	8	-	-	1
14.X.68	36	32	-	4	-	-	-
5.XI.68	56	51	-	3	-	1	-

PM= Peritrophic membrane

* Because the contents of the guts were often mixed, the totals for all food categories may exceed the number of animals in the sample.

TABLE XI

Gut contents of samples of Entomobryoides purpurascens collected in the Morgan Arboretum, Macdonald College, in 1969

Collection date	No. in sample	No. of specimens containing: Indet. debris	Pollen	Spores	Hyphae	Collemb. remains	* PM
13.IV.69	52	14	-	39	2	1	-
21.IV.69	52	29	-	24	4	3	-
30.IV.69	50	28	-	25	5	2	2
5.V.69	54	30	2	14	6	1	11
12.V.69	53	24	1	20	7	-	8
17.V.69	54	21	-	23	8	-	9

* Because the contents of the guts were often mixed, the totals for all food categories may exceed the number of animals in the sample.

PM= Peritrophic membrane

TABLE XII

Percentage mature males in samples of Entomobryoides purpurascens collected in the Morgan Arboretum, Macdonald College, in 1966-1967

Collection date	Total sample	Mature males	% males
17.XII.66	60	0	0
20.IV.67	128	0	0
15.VIII.67	38	9	23

TABLE XIII

Percentage mature males in samples of Entomobryoides purpurascens collected in the Morgan Arboretum, Macdonald College, in 1968.

Collection date	Total sample	Mature males	% males
19.I.68	66	0	0
25.III.68	50	0	0
15.IV.68	50	0	0
22.IV.68	50	0	0
29.IV.68	49	1	2
6.V.68	57	12	19
13.V.68	54	12	22
27.V.68	68	12	17.5
11.VI.68	44	9	20
25.VI.68	55	16	29
8.VII.68	55	14	25
8.IX.68	57	4	7
23.IX.68	55	0	0
30.IX.68	39	0	0
14.X.68	54*	0	0
5.XI.68	56	0	0

* Includes 20 specimens of which only the terminal abdominal segments were mounted and therefore no gut contents were recorded for these.

TABLE XIV

Percentage mature males in samples of Entomobryoides purpurascens collected in the Morgan Arboretum, Macdonald College, in 1969

Collection date	Total sample	Mature males	%males
13.IV.69	52	0	0
21.IV.69	52	0	0
30.IV.69	50	0	0
5.V.69	54	0	0
12.V.69	53	1	2
17.V.69	54	16	29

TABLE XV

Temperatures in the habitat of Entomobryoides purpurascens recorded in spot checks during 1968 (in the Morgan Arboretum, Macdonald College)

Date	Woodpile Temp. °C.	Air Temperature °C.
27.II.68	-6	-1
6.V.68	8	12
13.V.68	12	18
27.V.68	10	16
11.VI.68	14	17
25.VI.68	15	18
8.VII.68	20	23
22.VII.68	25	28
5.VIII.68	20	23
19.VIII.68	14	18
2.IX.68	19	24
18.IX.68	19	22
28.X.68	10	12

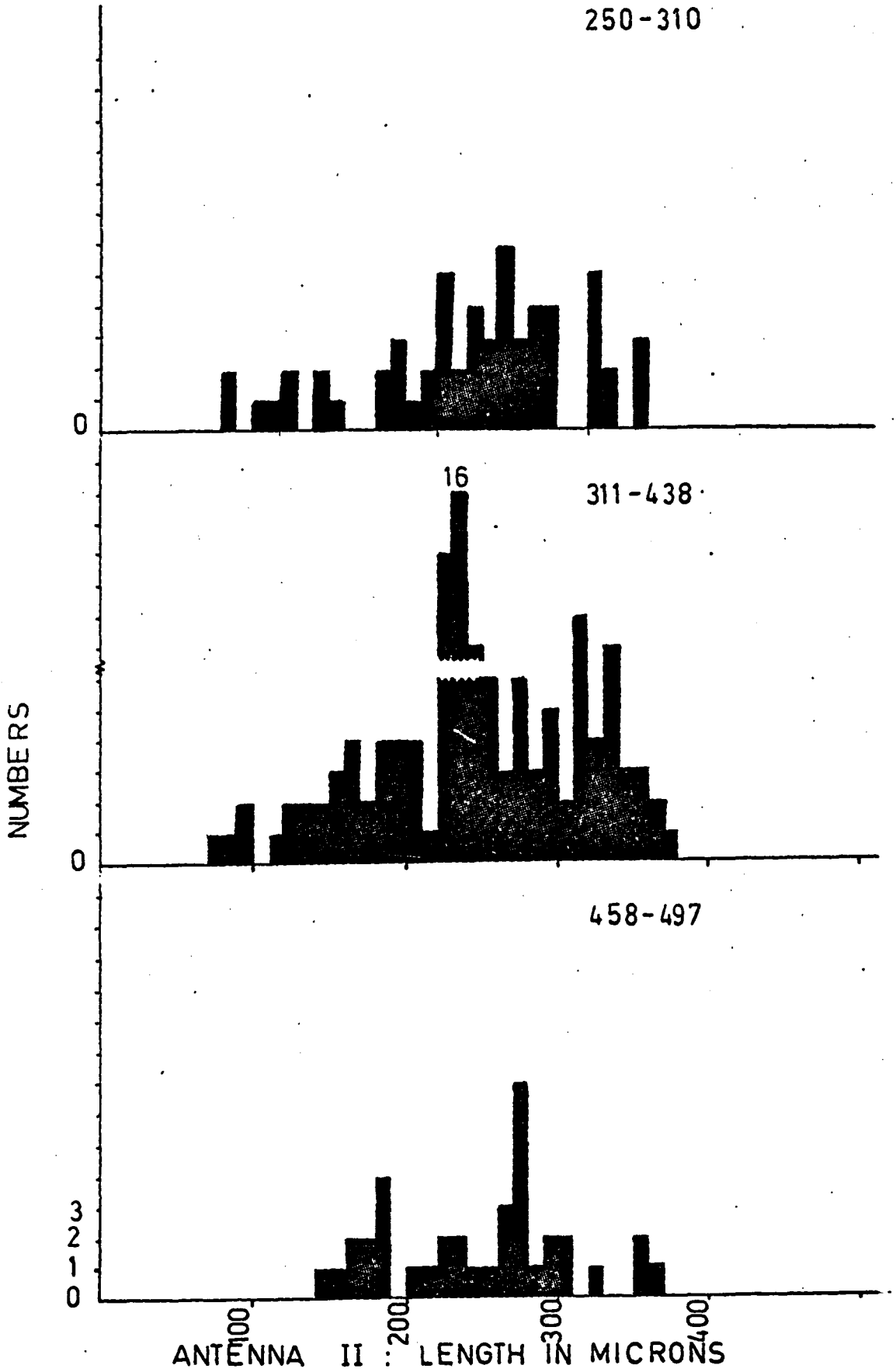
Figure 1

Entomobryoides purpurascens: frequency distribution of lengths of antennal segment II, in three samples collected in the Morgan Arboretum, Macdonald College as follows:

Upper- specimens 250-310, collected 17.XII.66

Middle- specimens 311-438, collected 20.IV.67

Lower- specimens 458-497, collected 15.VIII.67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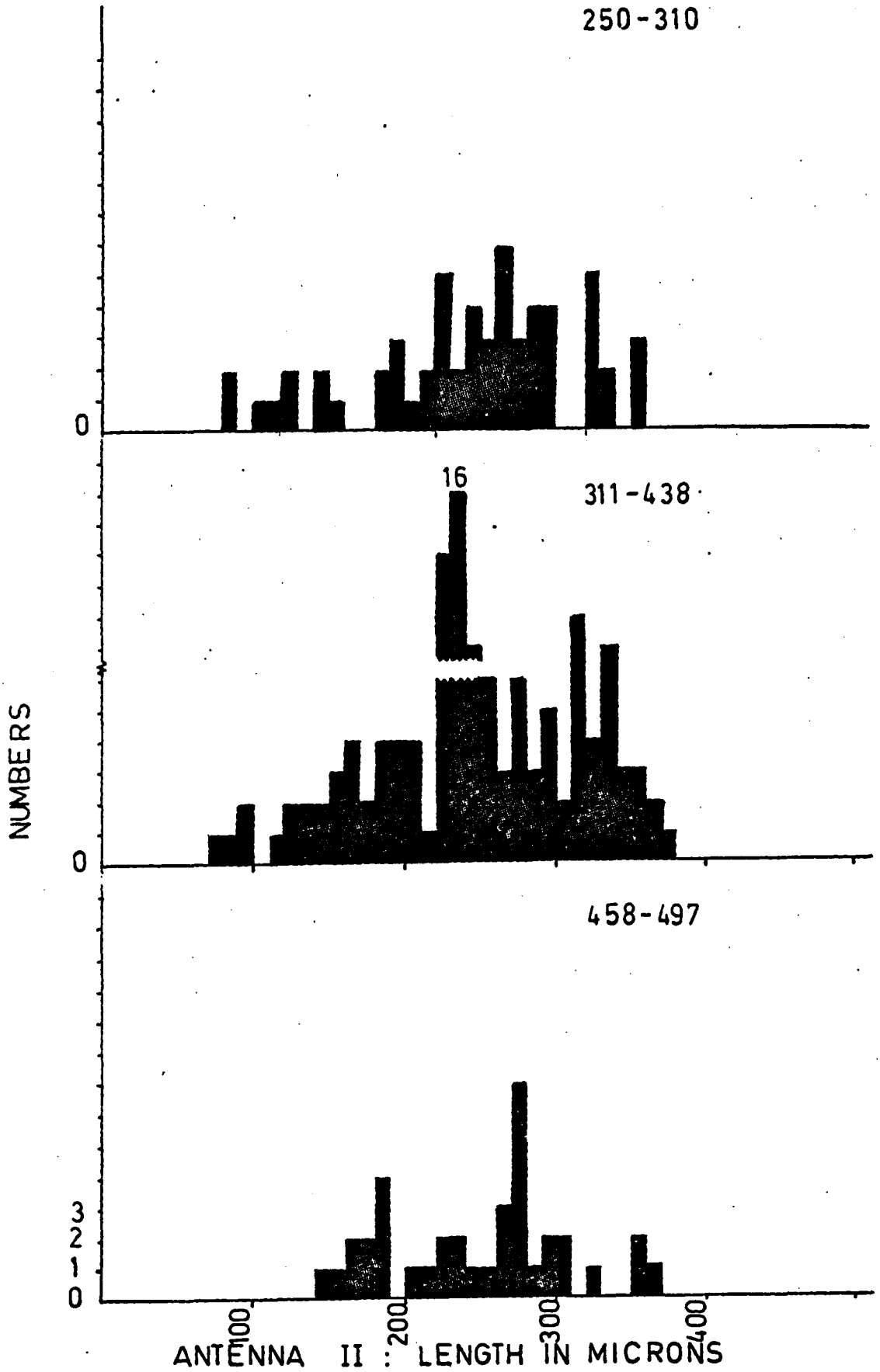
16
311-438
0
NUMBERS
458-497
3
2
1
0
100
200
300
400
ANTENNA II : LENGTH IN MICRONS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0
16
311-438
0
NUMBERS
458-497
3
2
1
0
100
200
300
400
ANTENNA II : LENGTH IN MICRONS

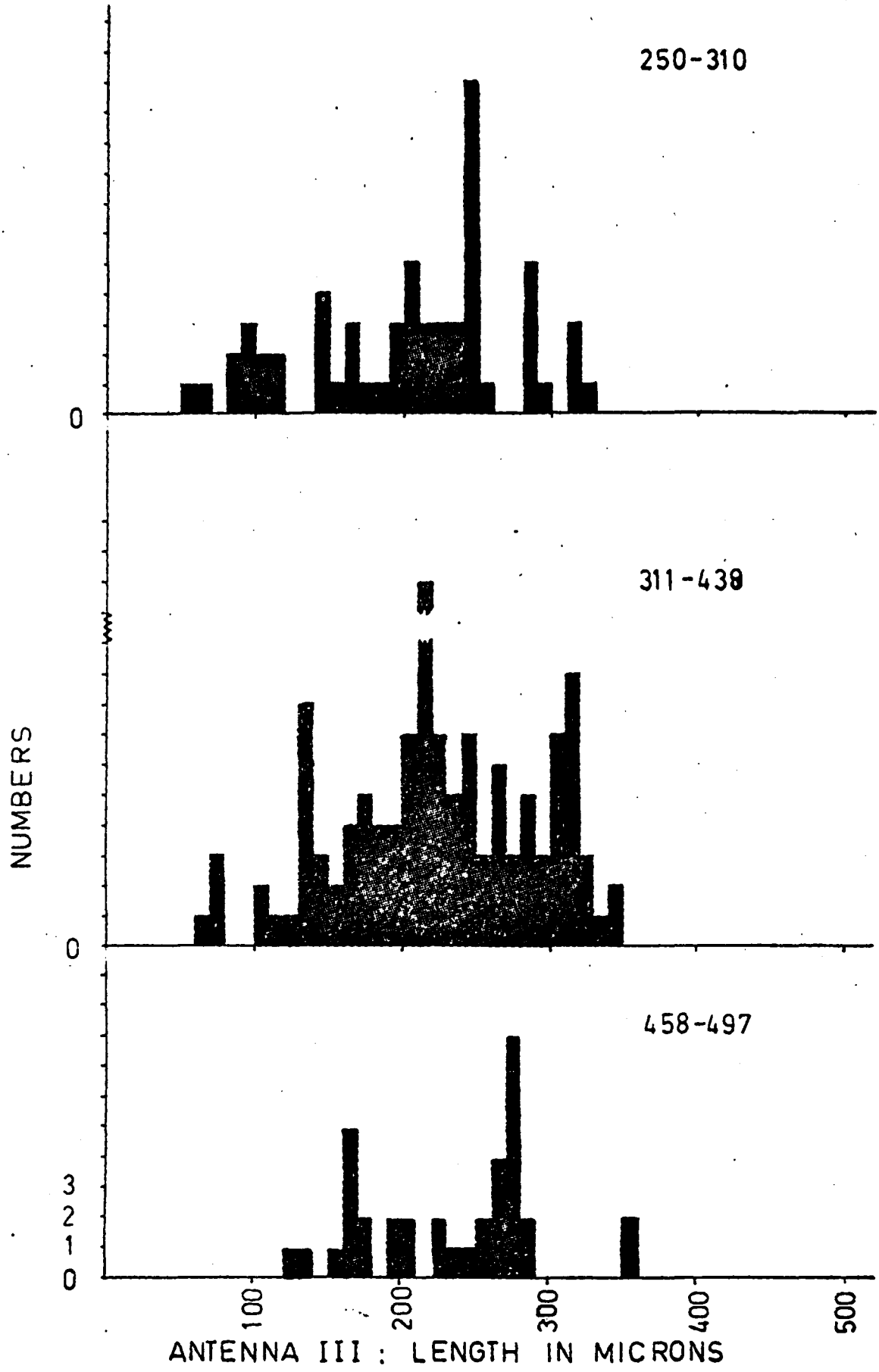
Figure 2

Entomobryoides purpurascens: frequency distribution of lengths of antennal segment III in three samples collected in the Morgan Arboretum, Macdonald College, as follows:

Upper- specimens 250-310, collected 17.XII.66

Middle- specimens 311-438, collected 20.IV.67

Lower- specimens 458-497, collected 15.VIII.67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0
311-438
0
458-497
3
2
1
0
NUMBERS
100
200
300
400
500
ANTENNA III : LENGTH IN MICRONS

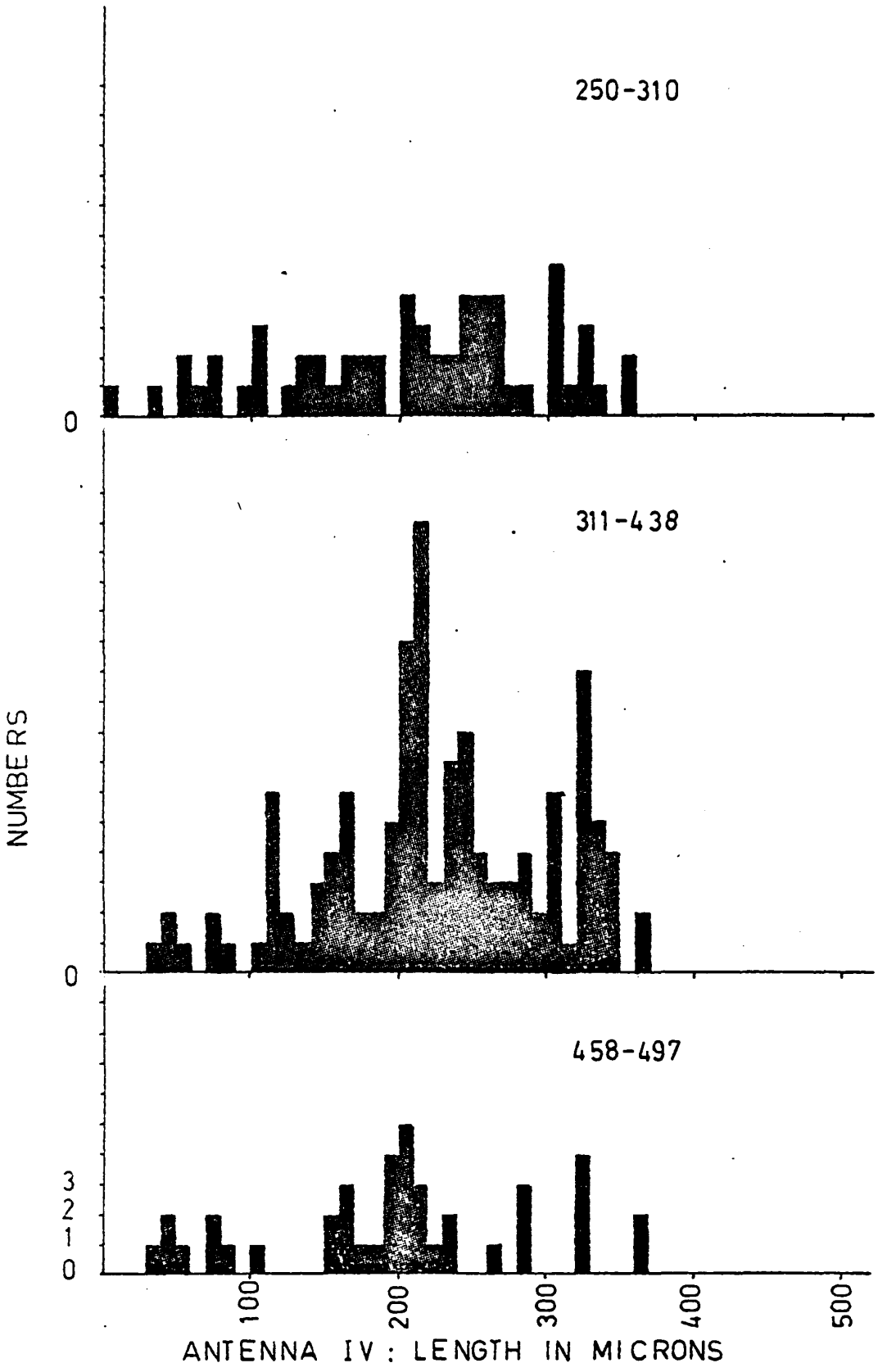
Figure 3

Entomobryoides purpurascens: frequency distribution of lengths of antennal segment IV in three samples collected in the Morgan Arboretum, Macdonald College, as follows:

Upper- specimens 250-310, collected 17.XII.66

Middle- specimens 311-438, collected 20.IV.67

Lower- specimens 458-497, collected 15.VIII.67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0
311-438
0
458-497
3
2
1
0
NUMBERS
100
200
300
400
500
ANTENNA IV : LENGTH IN MICRONS

Figure 4

Entomobryoides purpurascens: frequency distribution of lengths of femur I in three samples collected in the Morgan Arboretum, Macdonald College, as follows:

Upper- specimens 250-310, collected 17.XII.66

Middle- specimens 311-438, collected 20.IV.67

Lower- specimens 458-497, collected 15.VIII.67

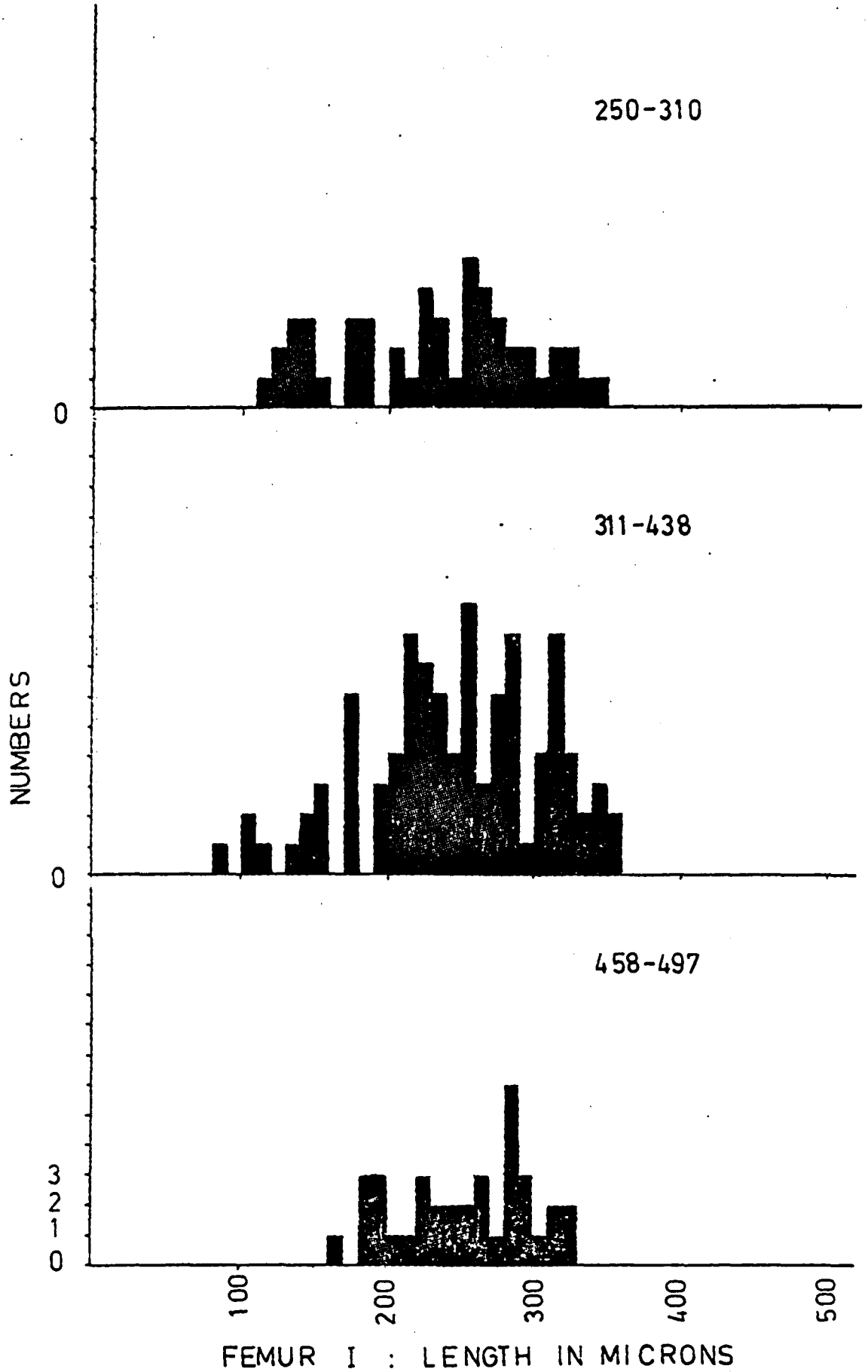

250-310
311-438
458-497
NUMBERS
0
0
3
2
1
0
100
200
300
400
500
FEMUR I : LENGTH IN MICRONS

Figure 5

Entomobryoides purpurascens: frequency distribution of lengths of tibiotarsus I in three samples collected in the Morgan Arboretum, Macdonald College, as follows:

Upper- specimens 250-310, collected 17.XII.66

Middle- specimens 311-438, collected 20.IV.67

Lower- specimens 458-497, collected 15.VIII.67

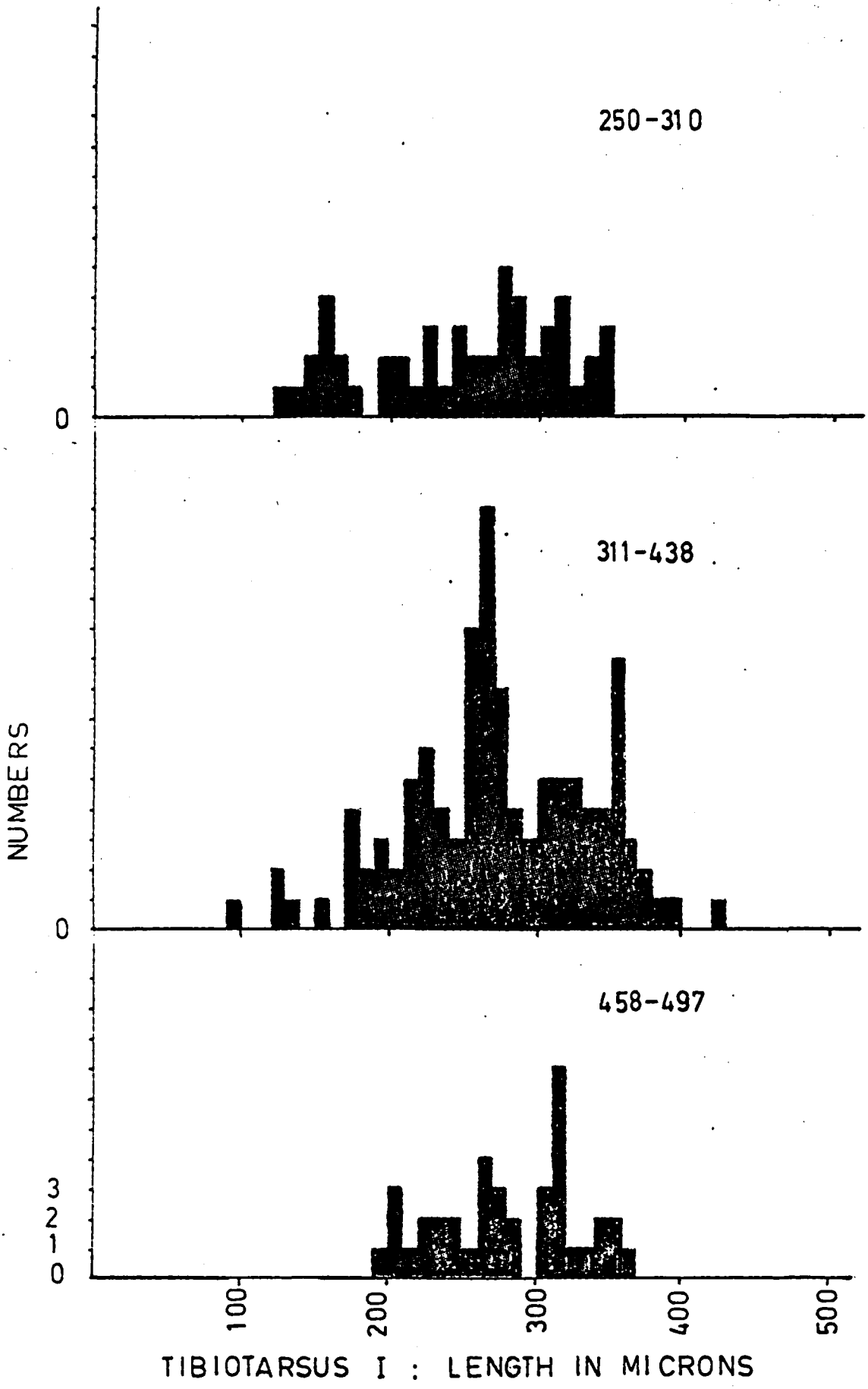

250-310
311-438
458-497
NUMBERS
0
0
3
2
1
0
100
200
300
400
500
TIBIOTARSUS I : LENGTH IN MICRONS

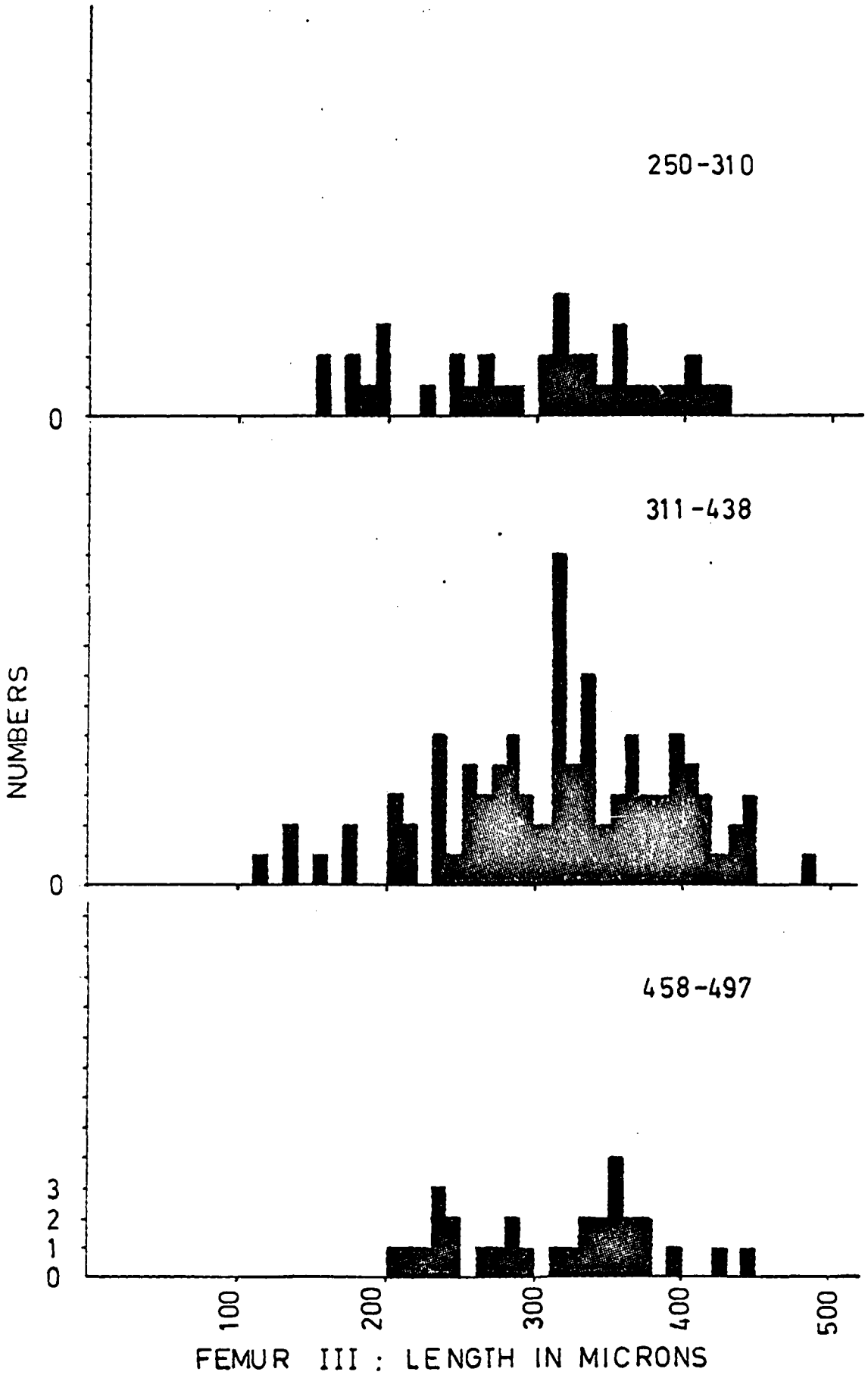
Figure 6

Entomobryoides purpurascens: frequency distribution of lengths of femur III in three samples collected in the Morgan Arboretum, Macdonald College, as follows:

Upper- specimens 250-310, collected 17.XII.66

Middle- specimens 311-438, collected 20.IV.67

Lower- specimens 458-497, collected 15.VIII.67


250-310
311-438
458-497
NUMBERS
0
0
3
2
1
0
100
200
300
400
500
FEMUR III : LENGTH IN MICRONS

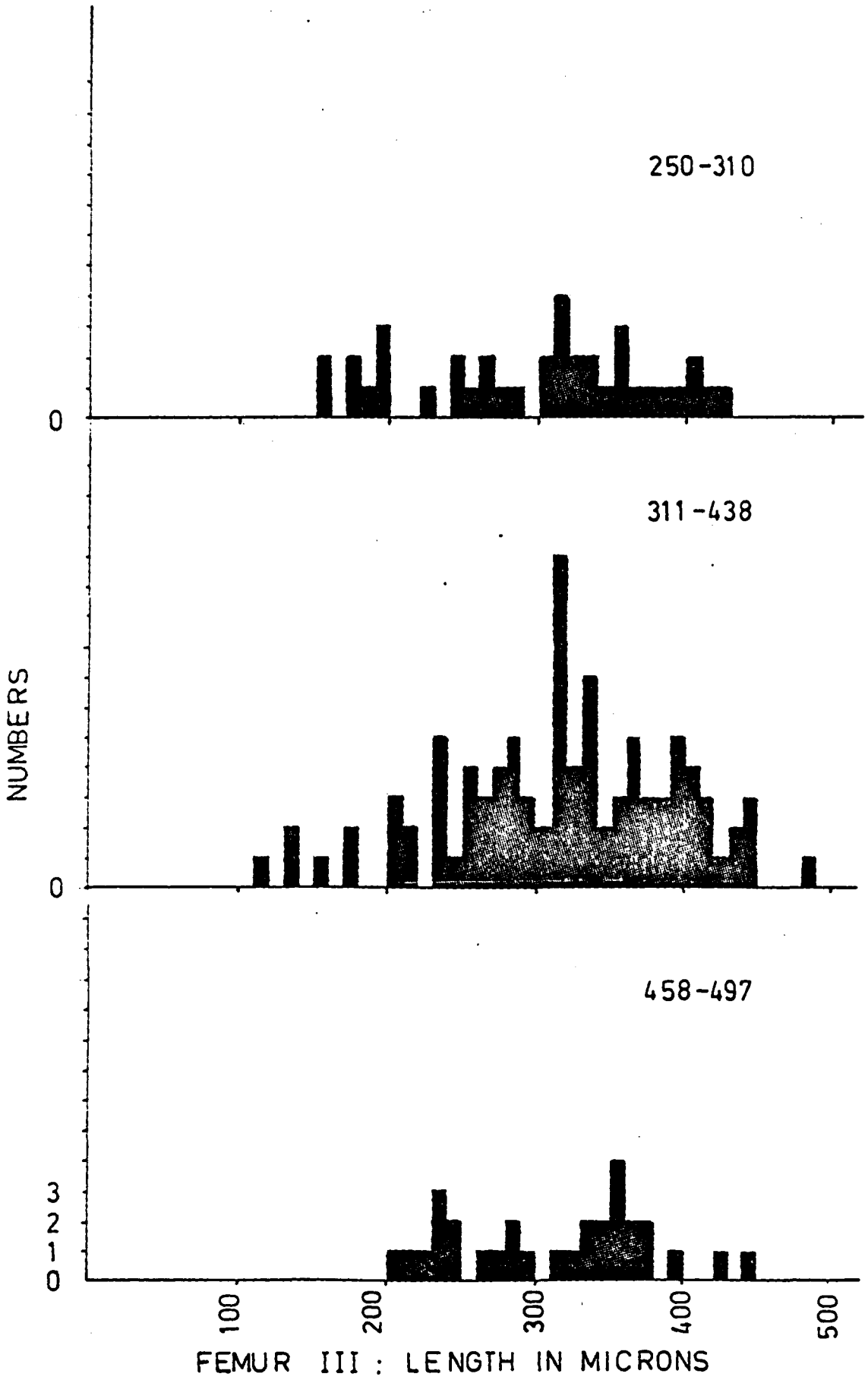

250-310
311-438
458-497
NUMBERS
0
0
3
2
1
0
100
200
300
400
500
FEMUR III : LENGTH IN MICRONS

Figure 7

Entomobryoides purpurascens: frequency distribution of lengths of tibiotarsus III in three samples collected in the Morgan Arboretum, Macdonald College, as follows:

Upper- specimens 250-310, collected 17.XII.66

Middle- specimens 311-438, collected 20.IV.67

Lower- specimens 458-497, collected 15.VIII,67

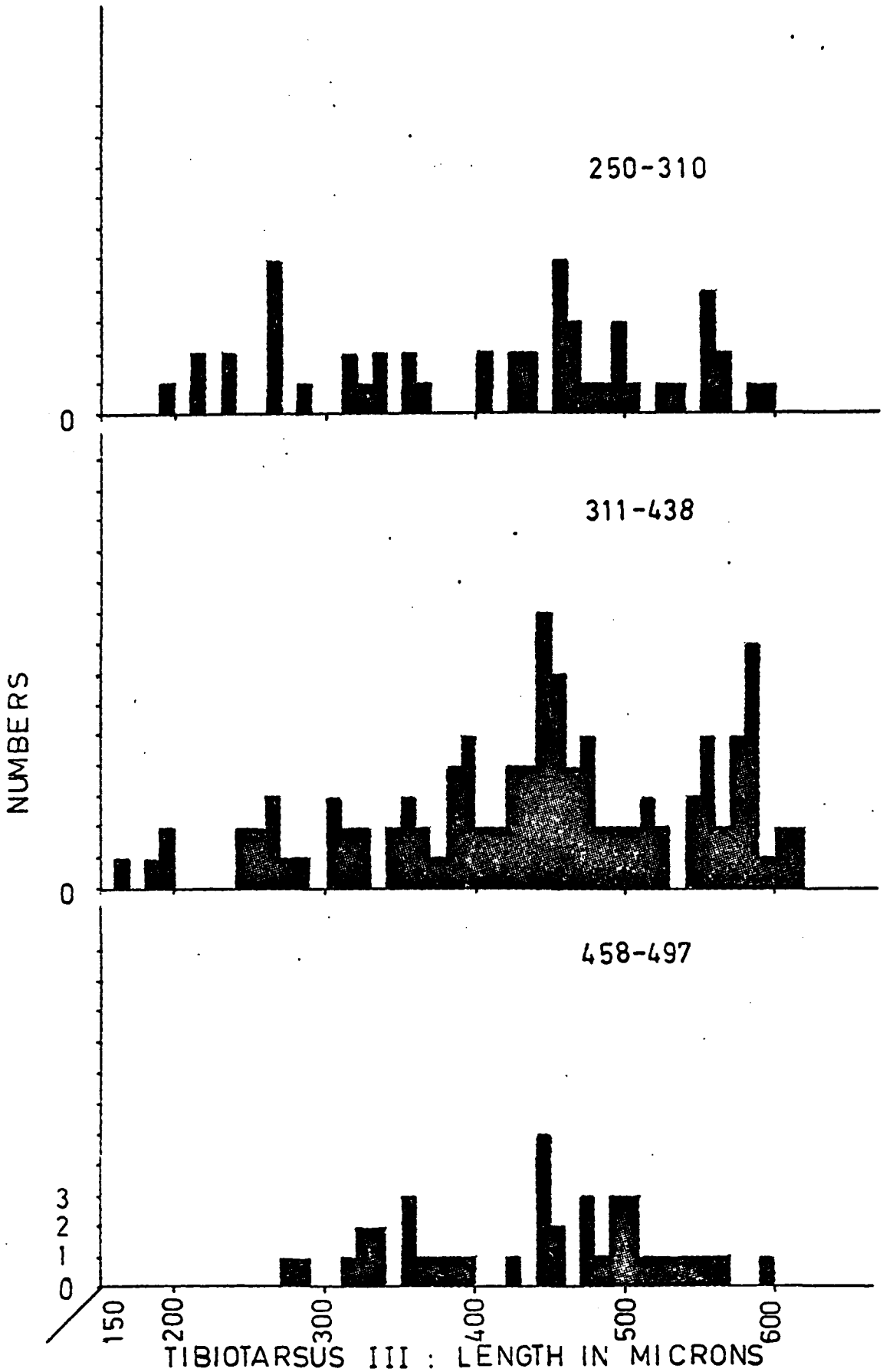

250-310
311-438
458-497
NUMBERS
0
0
3
2
1
0
150
200
300
400
500
600
TIBIOTARSUS III : LENGTH IN MICRONS

Figure 8

Entomobryoides purpurascens: frequency distribution of lengths of unguifer III in three samples collected in the Morgan Arboretum, Macdonald College, as follows:

Upper- specimens 250-310, collected 17.XII.66

Middle- specimens 311-438, collected 20.IV.67

Lower- specimens 458-497, collected 15.VIII.67

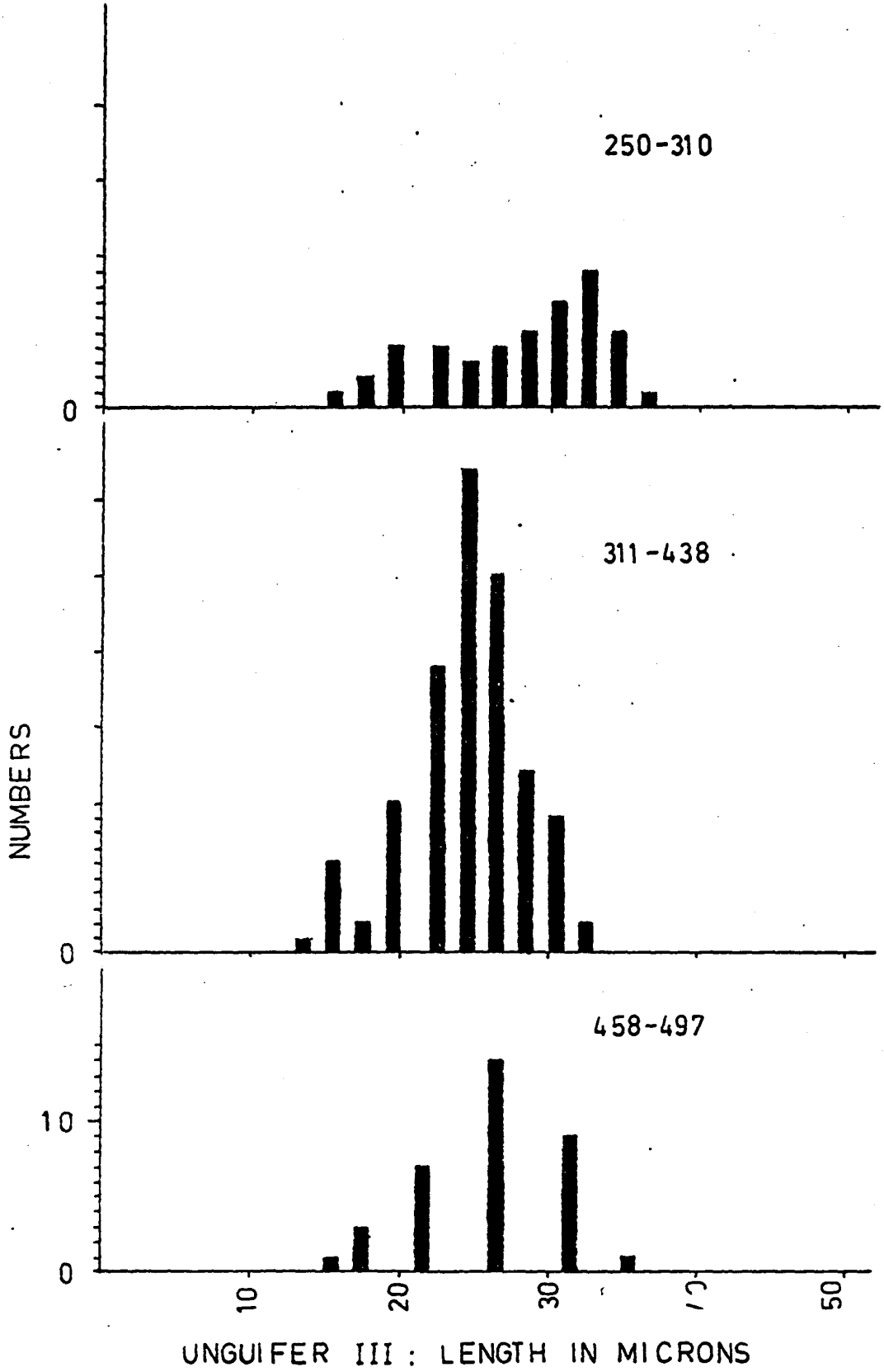

250-310
311-438
458-497
0
0
10
0
NUMBERS
10
20
30
50
UNGUIFER III : LENGTH IN MICRONS

Figure 9

Entomobryoides purpurascens: frequency distribution of lengths of unguis III in three samples collected in the Morgan Arboretum, Macdonald College, as follows:

Upper- specimens 250-310, collected 17.XII.66

Middle- specimens 311-438, collected 20.IV.67

Lower- specimens 458-497, collected 15.VIII.67

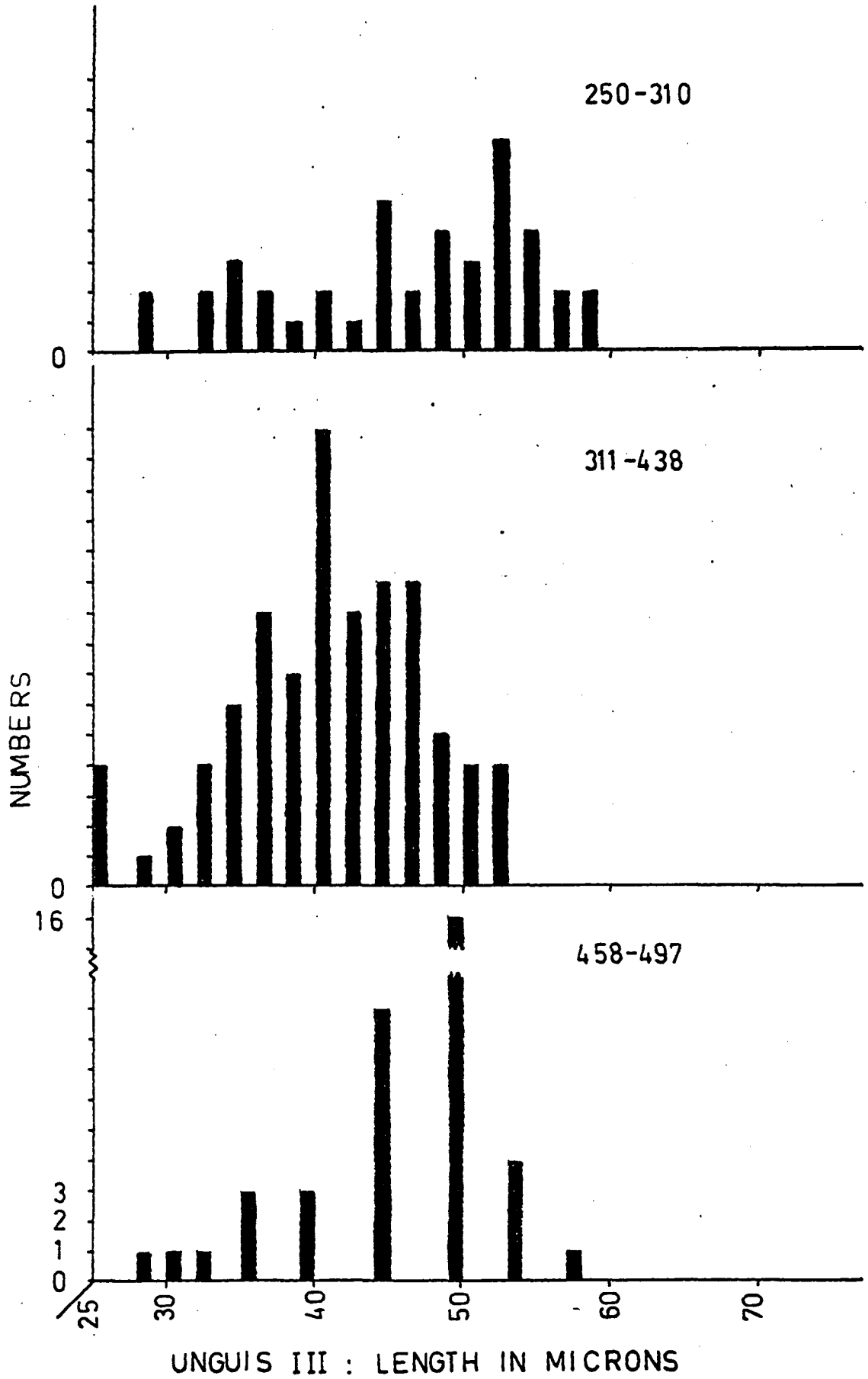

250-310
311-438
458-497
NUMBERS
0
0
16
3
2
1
0
25
30
40
50
60
70
UNGUIS III : LENGTH IN MICRONS

Figure 10

Percentage of mature males in samples of Entomobryoides purpurascens collected in the Morgan Arboretum, Macdonald College, in 1968.

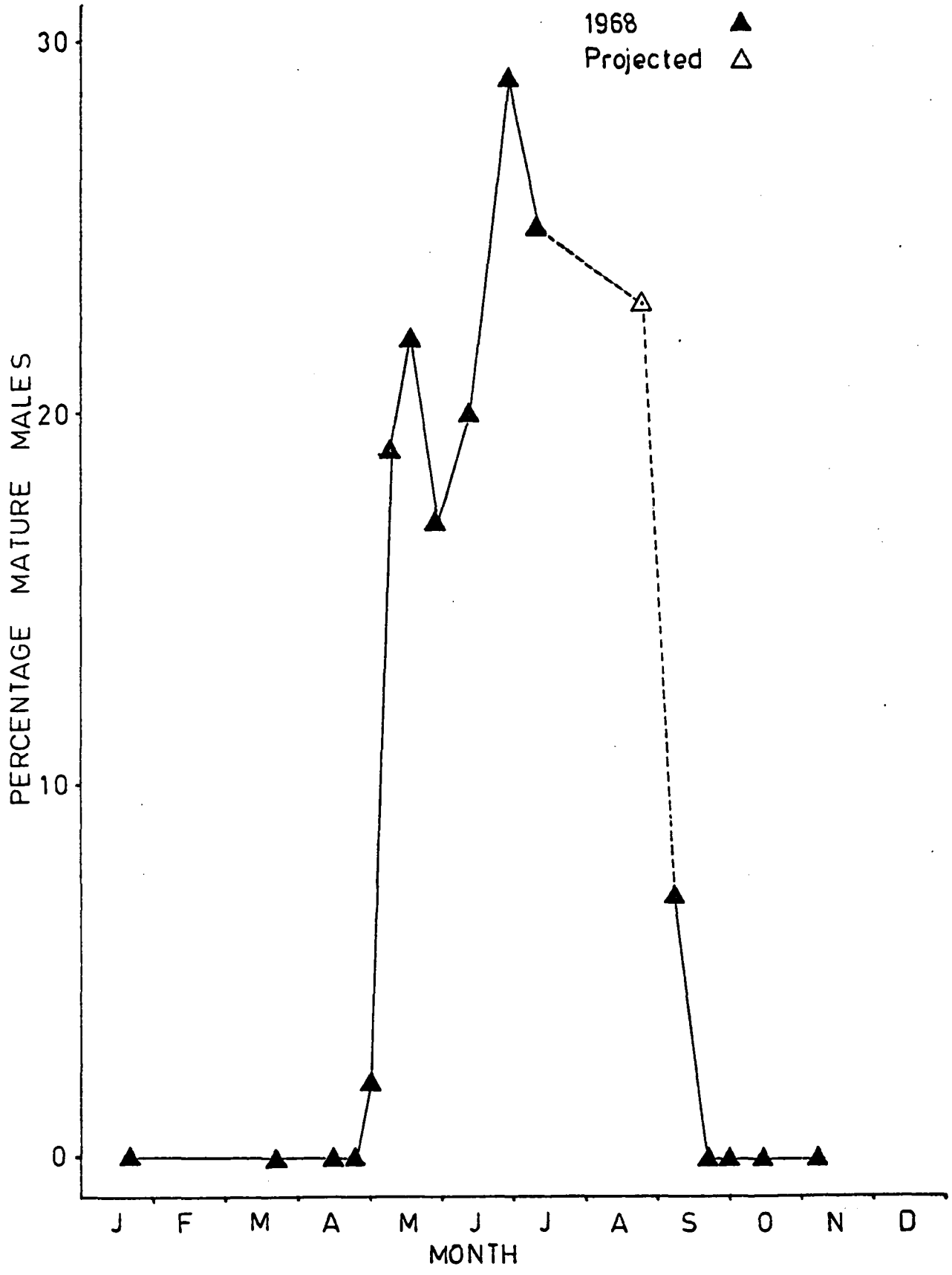

1968
Projected
30
20
10
0
PERCENTAGE MATURE MALES
J
F
M
A
M
J
J
A
S
O
N
D
MONTH

Figure 11

A comparison of spring changes in percentage of males in samples of Entomobryoides purpurascens collected in the Morgan Arboretum, Macdonald College, in 1968 and 1969.

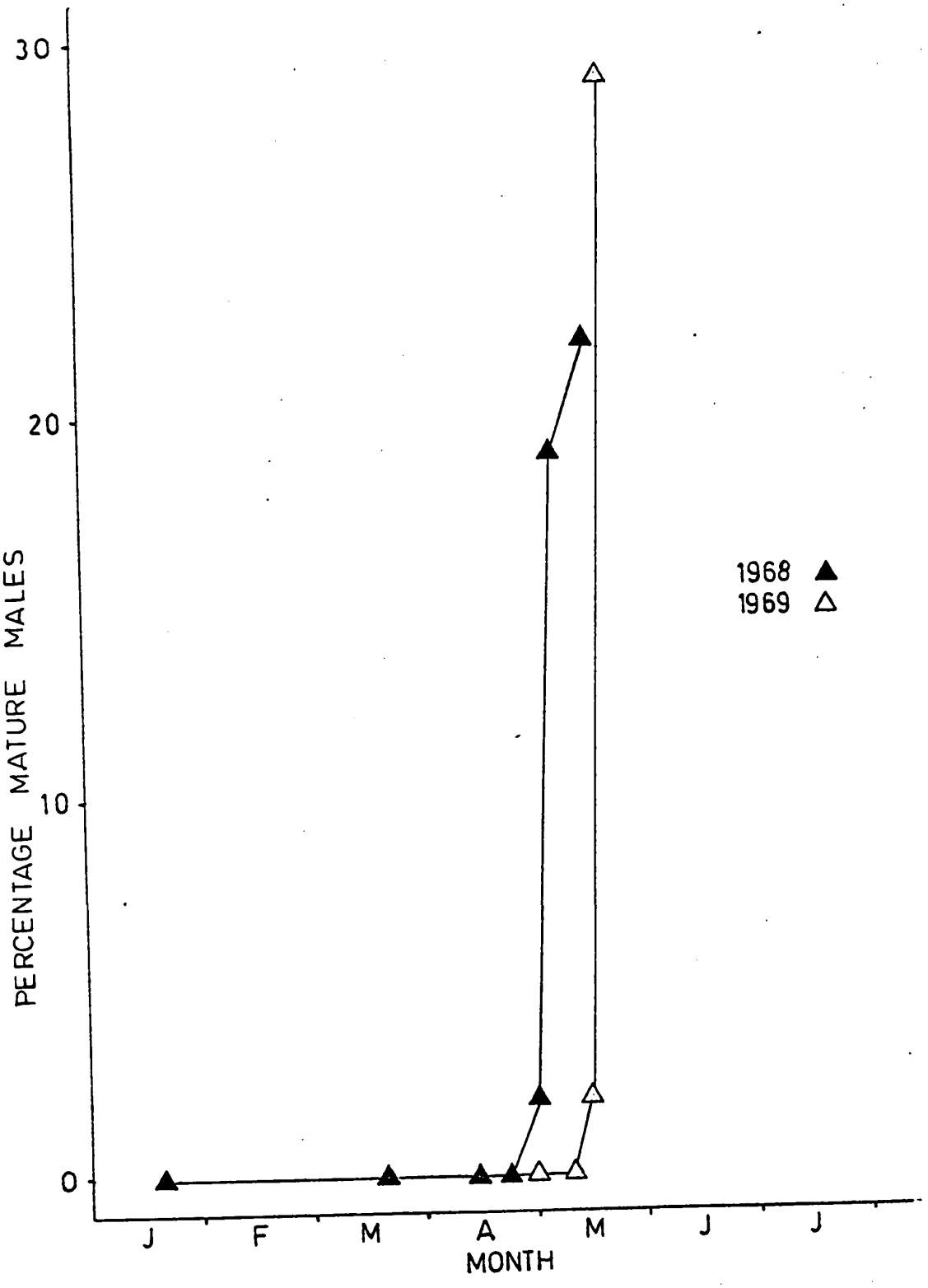

30
20
10
0
PERCENTAGE MATURE MALES
1968
1969
J
F
M
A
M
J
J
MONTH

Figure 12

Interval between collection and oviposition when Entomobryoides purpurascens reared at 25°C. and $15^{3}/4$ hour photophase from date of collection.

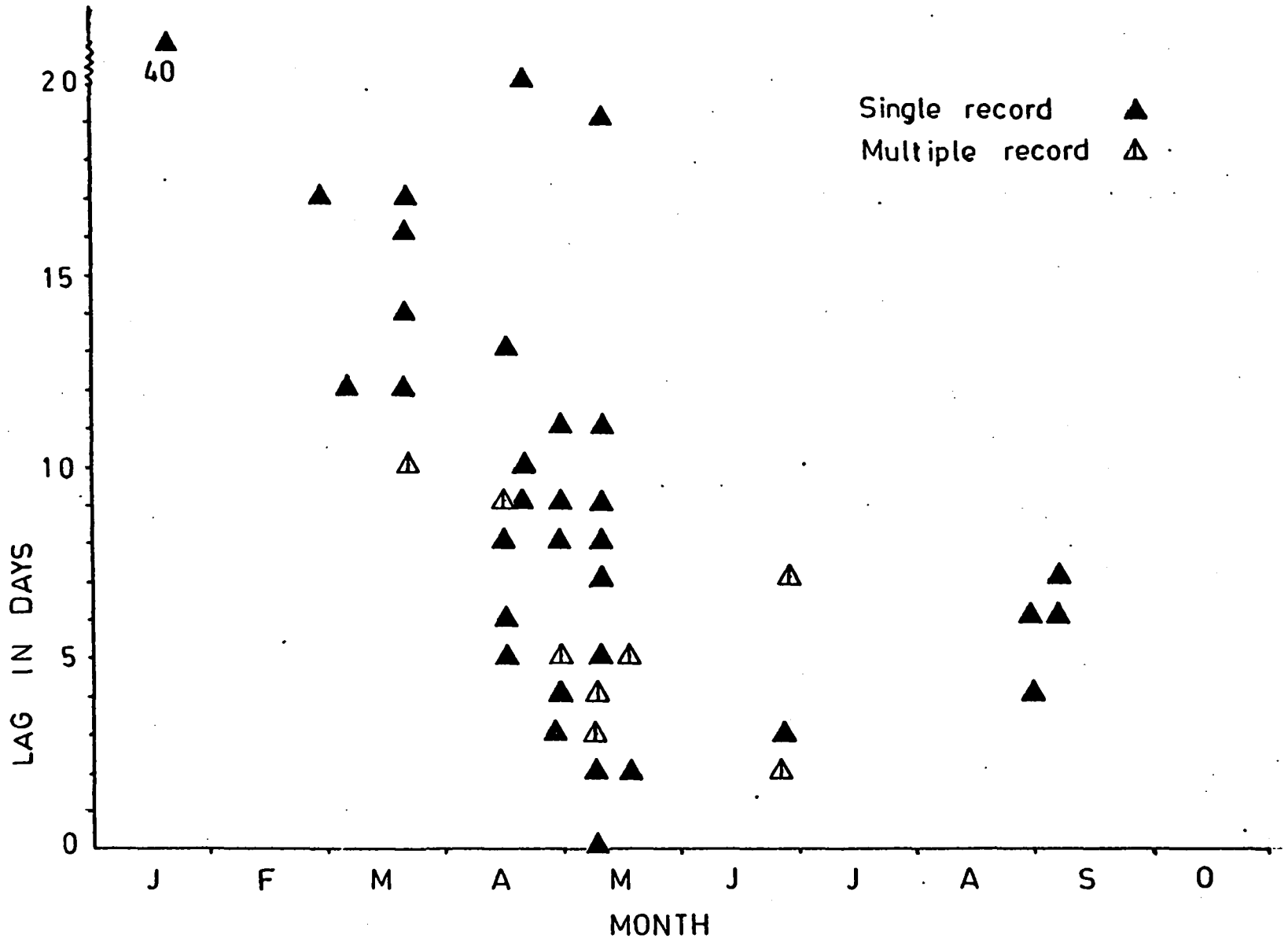

40
20
15
10
5
0
LAG IN DAYS
Single record
Multiple record
J
F
M
A
M
J
J
A
S
O
MONTH

Figure 13

Egg of Entomobryoides purpurascens showing one half of the chorion and the characteristic ridges in the serosal membrane.

Magnification 200x approx.