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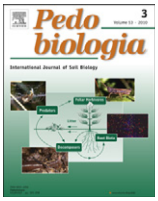

Life cycle and reproductive traits of the earthworm *Aporrectodea trapezoides* (Dugès, 1828) in laboratory cultures

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A B S T R A C T

The parthenogenetic earthworm *Aporrectodea trapezoides* (Dugès, 1828) is widely distributed all over the world due to European agricultural practices. In order to provide baseline life cycle data, cocoons were obtained from field-collected individuals and their features and viability, incubation period, number of hatchlings and mortality rate were recorded. Singleton and twin earthworms from this first experiment were cultured from hatching during a 490-day period under controlled conditions with biomass, survival, reproductive condition and cocoon production recorded at intervals of 15 days. On average, individuals of isolated-reared *A. trapezoides* reached maturity at day 153 and body weight at maturity was approximately 1 g. In order to record reproductive traits and differences between field-collected and laboratory-reared individuals, 40 microcosms with an isolated earthworm (20 with field-collected individuals and 20 with laboratory-reared ones) and 40 containing groups of three (20 with each type of individual) were maintained during a complete year under controlled conditions. The amount of soil per individual was the same in both types of microcosm. Both the individuals kept in isolation and those cultured in groups produced cocoons, hence completely proving the obligatory parthenogenetic reproduction in this species without copulation or need of any physical–chemical stimulus. In general, isolated earthworms produced a significantly higher number of cocoons than those in groups of three, and the same was recorded for laboratory-reared earthworms when compared with field-collected ones. This study highlights the importance of knowing the life cycle and reproductive traits of one possible key species in soil management due to its vast distribution and high density in soils, and the species' highly recommended use in applied studies because of its ease of culture.

Introduction

Aporrectodea trapezoides is a medium-sized earthworm of Palaearctic origin but almost worldwide distribution. Although typically a Holarctic species, it has been introduced in Australia (Blakemore, 1999), New Zealand (Fraser et al., 1996), South Africa (Reinecke and Ljungstrom, 1969) and both North (Zaitlin et al., 2007) and South America (De Mischis, 1997). These introductions were due to the spread of European agricultural practices and widespread use of this earthworm as fishing bait (Blakemore, 2006). Bouché (1972) defined this species as anecic, thus living in vertical galleries in the soil.

All the earthworms used in this study were morphologically and anatomically identified as *A. trapezoides*, following the taxonomic key of Gates (1972). Specifically, the position of genital setal tumescence (in segments 9, 10 and 11), the location of the clitellum in (25–26) 27–34 (35), tubercula pubertatis (undivided in segments 31–33) and male sterility (empty spermathecae and male funnels, both in segments 10 and 11) were taken into account in identifying the earthworms.

In other earthworm species, such as *Octolasion tyrtaeum* (Muldal, 1952; Jaenicke and Selander, 1979), male structures are not reduced and pseudogamy – individuals copulate in order to exchange spermatophores that are invariably empty – is prevalent. Thus, although spermatozoids are not necessary, this species needs some mechanical or chemical stimulus to trigger reproduction.

Polymorphic degradation of reproductive structures, as often found in other parthenogens, has not yet been recognized in this species. Both primary and secondary male sexual characters such as perithecal papillae, tubercula pubertatis, spermathecae, swollen male porophores and seminal vesicles are retained in *A. trapezoides*. This fact led Gates (1972) to pose two possible hypotheses: the recent appearance of parthenogenesis or the unusual resistance to mutation in those organs. Gates (1972) also suggested that in *A. trapezoides*, pseudogamy could have evolved into male sterility. As neither reproduction in this species has been well studied nor pseudogamy, the need for stimulation to trigger its reproduction have never been proven.

Traditionally, only little attention has been paid to life cycle of parthenogenetic earthworms (ex: *Octolasion cyaneum*, Lowe and

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Butt, 2008; *Perionyx ceylanensis*, Karmegan and Daniel, 2009). Nevertheless, earthworm species with this kind of reproduction could be potentially more appropriate than hermaphroditic ones in applied studies. As suggested by Lowe and Butt (2007), obligatory parthenogenetic species should be preferrable to amphimictic species as ecotoxicological test organisms due to their low genetic heterogeneity. Also, these earthworms can be maintained in isolation, allowing for individually monitored endpoints. Experiments on life cycles have usually been done only with field-collected individuals or with laboratory-reared ones. Hence, some aspects have been poorly tested, such as the existence of putative learning processes in the field, and differences between potential and real growth and reproduction.

Interest in *A. trapezoides* has been strongly biased towards its role in soils. Some ecological aspects, such as its distribution as an exotic species and its effect on soil properties, crops or farming systems, have been considered very important. Nevertheless, its life cycle has only been studied under field conditions (population dynamics and changes in abundance) and during short periods of time (19 weeks; McCredie et al., 1992). Thus, little is known about the life cycle of one of the most abundant earthworm species distributed worldwide. This fact hinders the development of appropriate management strategies to optimise its role in maintaining soil properties and in assessing its potential effects on natural and agricultural ecosystems in relation to its possible use in applied studies of ecotoxicology, vermicomposting or ecological restoration.

Therefore, the aims of this study were to obtain baseline data on: (1) cocoon and hatchling production, morphological features and viability; (2) growth rates under optimal laboratory conditions; (3) possible requirements to trigger reproduction; and (4) differences in reproduction between field-collected individuals and laboratory-reared ones.

Materials and methods

Cocoon features and number of hatchlings from field-collected individuals

Mature individuals of *A. trapezoides* were obtained by digging and hand sorting of soil from a grassland in El Molar (Madrid, Spain, 40° 44′22.9 N; 003° 33′53.1 W). Earthworms were kept in two plastic vessels (between 15 and 25 per vessel, surface area 0.19 m^2, depth 10 cm) with sealable lids pierced with a needle to allow ventilation. These microcosms were filled with 2 kg of field-collected soil previously dried and sieved (2 mm). The earthworms were fed dried cow manure that was added to the soil surface (1% of soil weight) and they were kept in a scientific incubator at 16 °C in 24 hours darkness. Soil moisture was maintained at 25% (previously stated to be in the optimal range of moisture percentage for several lumbricid species; Lowe and Butt, 1999). Cocoons (n=244) were hand-sorted every 15 days from these stocks, their size and weight were recorded, and 147 of them were incubated in separately labelled Petri dishes on filter paper (Whatman No. 1) with excess water to avoid desiccation. A total of 241 hatchlings were obtained. Dishes were inspected daily in order to record viability and number of hatchlings per cocoon. Length of incubation was calculated as the time to hatch after collection plus 7 days (half of the time between sampling periods).

Growth of laboratory-reared individuals

Thirty-seven of the 241 hatchlings (24 singletons and 13 twin individuals) were maintained individually in 250 ml plastic vessels (surface area 0.025 m^2, depth 5.5 cm) with 250 g of dried and sieved soil (2 mm), fed with dried cow manure (1% of soil weight), with 25% moisture and maintained at 16 °C. Individual earthworm survival, biomass and reproductive condition were recorded every 15 days for a 490-day period. Vessel contents were replaced at each sampling. When the individuals reached maturity (defined by the presence of a swollen clitellum), cocoons were collected by wet-sieving the soil (2 mm). A two-sample Student's t-test was employed to analyse differences in weight and total cocoon production between singletons and twins. An alpha level of $P \leq 0.05$ was considered statistically significant. All analyses were completed using the software Statistica version 6.0. Data are presented as means ± S.D.

As a complementary growth measure, instantaneous growth rates (IGRs) were calculated for each 15-day growth interval. IGRs assume that growth proceeds logistically rather than linearly (Eriksen-Hamel and Whalen, 2006). Their values are obtained by calculating the change in an individual's growth during an infinitely short time interval (Pertrusewicz and Macfayden, 1970). The IGR was calculated using the equation:

$$\mathrm{IGR} = \ln(W_f/W_i)/\Delta t$$

where W_i and W_f are initial and final earthworm mass (g), respectively, and Δt is the growth interval measured in days (Brafield and Llewellyn, 1982).

Detecting possible requirements to trigger parthenogenetic reproduction

Mature individuals (n=40) collected during different seasons of the year (autumn, winter and spring) in El Molar were studied anatomically to check for the absence of sperm in the spermathecae. These organs were removed, placed on a slide, squashed under a cover slip, and viewed under a light microscope (400X). Once male sterility was checked in this population, 80 mature individuals were field-collected to prepare the experiment. Twenty of these were kept individually in 250 ml plastic vessels (labelled as *If*). Sixty individuals were maintained in groups of three in 750 ml plastic vessels (labelled as *Gf*; surface 0.041 m^2, depth 12.5 cm). The contents and conditions of each microcosm were the same as in experiment 2 (see above), except with three times more soil in the second type of microcosm to maintain a constant quantity per earthworm. Every 15 days, vessel contents were replaced. The discarded soil was wet-sieved in order to collect cocoons. This experiment was maintained over an entire year. The same procedures were undertaken with laboratory-reared individuals, with the microcosms containing one individual labelled *Il* and those with three earthworms, *Gl*.

Repeated measures analyses of variance (RM-ANOVA), followed by post-hoc paired comparison t-tests (when appropriate) were employed to test for significant differences. Mauchley's test was conducted to examine sphericity for the repeated measures analyses. If the assumption of sphericity was violated, the Greenhouse-Geisser adjustment was used to protect against type I error (Keselman et al., 2001). In every case, the significance level was set at 5%.

Results

Cocoon features and number of hatchlings from field-collected individuals

A. trapezoides produced between 1 and 4 hatchlings per cocoon, with 87% of 244 collected cocoons hatching successfully. Of these, 59% yielded singletons and 38% twins. Remarkably, 3 cocoons produced triplets (2%) and 2 produced quadruplets

(1%). The mean biomass was 0.014 ± 0.006 g, ranging from 0.0011 g (in quadruplets) to 0.0327 g (in singletons) (n=241). The mean obtained from this data was 1.67 ± 0.59 hatchlings per cocoon. In 2% of the cocoons, conjoined twin individuals (at head or tail) were recorded. During the first two weeks, 4.9% of twins and 1.69% of singletons died. One month after hatching, hatchling survival was 94.6%. Cocoons were characterised by a mean mass of 0.033 ± 0.008 g and a size of 5.7 ± 0.8 mm (length) and 3.3 ± 0.4 mm (width). Incubation time of these cocoons was 41.9 ± 9.6 days under culture conditions.

Growth of laboratory-reared individuals

After the 490-day experimental period, 100% survival of laboratory-reared individuals was recorded. The growth of *A. trapezoides* followed a sigmoid curve (Fig. 1), with a slow initial phase over 60 days (mean weight of 0.101 ± 0.026 g), an exponential phase over 285 days (mean weight of 2.745 ± 0.566 g), and a stationary growth phase reached after 345 days, with a mean weight of 3.307 ± 0.695 g. The length of growth phases was determined by calculating the points where both the lower and upper asymptotes cross the tangent line to the inflexion point. Sexual maturity was reached between days 142 and 165 both for singletons and twins, with a mean weight of 1.005 ± 0.172 g. Cocoon production was recorded as soon as maturity was reached, and the mean reproduction rate was 4.06 ± 1.26 cocoons per earthworm per two weeks (that is, approximately 105 cocoons per earthworm per year). Upon sampling, two of the individuals were found to be sporadically inactive and coiled in spherical chambers.

Significant differences between individual mean mass of singletons and twins were only recorded at days 1 and 15, with singletons having greater biomass (Student's t-test; t=3.48; df=36, $P<0.01$; t=3.51, df=36, $P<0.01$, respectively). No significant differences were observed in mean weight between either type of individuals during the remainder of the experiment (Student's t-test; $t=-0.23$, df=64, P=0.82), or in their mean reproductive rate (Student's t-test; t=1.01, df=36, P=0.317).

IGR values followed a logarithmic pattern, with the same phases observed as in the previous growth pattern (Fig. 2). During the exponential phase, IGR reached its lowest value at day 150, the same period when individuals reached sexual maturity. After day 330, IGR values were close to zero.

Detecting possible requirements to trigger parthenogenetic reproduction

All individuals of *A. trapezoides* kept in isolation (from both origins) produced cocoons throughout the experiment. All

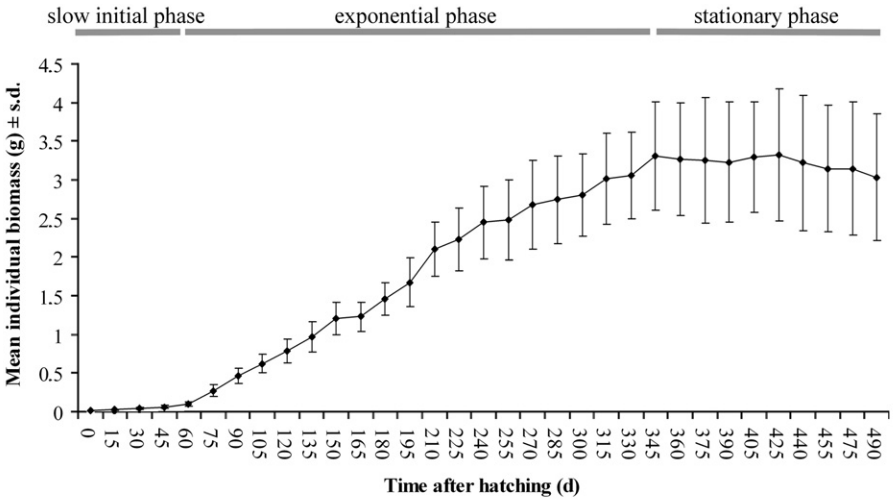

Fig. 1. Growth pattern in *Aporrectodea trapezoides* at 16 °C (means ± SE, n=37). Initial, exponential and stationary phases of growth are noted.

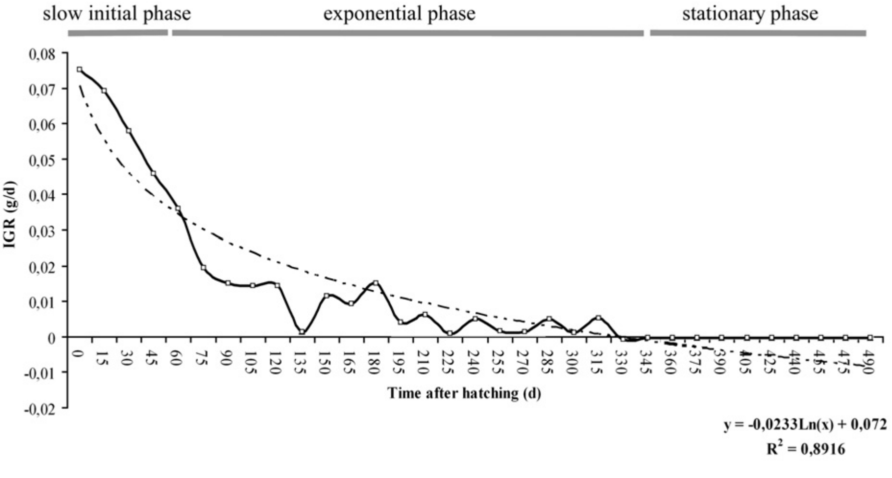

Fig. 2. Instantaneous Growth Rate values in *Aporrectodea trapezoides* at 16 °C (n=37). The trend line is showed as a dash line. Initial, exponential and stationary phases are noted.

Table 1
Significance of differences in the mean rate of cocoon production compared among the different types of microcosms.

Microcosms compared	*F*- and *P*-value	Significance of differences
If vs *Gf*	$F_{(1,19)}=18.23$; $P=0.183$	n.s.
Il vs *Gl*	$F_{(1,19)}=23.03$; $P<0.001$	* (> in *Il*)
Il vs *If*	$F_{(1,19)}=54.96$; $P<0.001$	* (> in *Il*)
Gl vs *Gf*	$F_{(1,19)}=34.42$; $P<0.001$	* (> in *Gl*)
(*If* + *Il*) vs (*Gf* + *Gl*)	$F_{(1,19)}=6.99$; $P<0.001$	* (> in *If* + *Il*)
(*If* + *Gf*) vs (*Il* + *Gl*)	$F_{(1,19)}=18.88$; $P<0.001$	* (> in *Il* + *Gl*)

n.s. indicates no significant differences while * indicates significant differences. In brackets, the microcosm type in which the highest cocoon production was recorded for each comparison is noted.

individuals collected in the field during every season of the year were dissected to check male sterility, hence pseudogamy was ruled out and parthenogenetic reproduction was proven in this population. Nevertheless, cocoon production was different in the four types of microcosms, as detailed below.

The mean rate of cocoon production per earthworm per 15 days was compared among the different microcosms (*If*, *Gf*, *Il* and *Gl*). Mean cocoon production throughout the year in each kind of microcosm was 1.60 ± 0.83 cocoons per earthworm per 15 days in *If*, 1.31 ± 0.47 in *Gf*, 4.16 ± 1.49 in *Il* and 2.44 ± 1.71 in *Gl*. Repeated measures one-way ANOVA showed no significant differences in rates between *If* and *Gf* microcosms, but cocoon production rates were significantly higher in earthworms from *Il* microcosms than those from *Gl* ones, in individuals from *Il* microcosms compared to those from *If* ones, and in specimens from *Gl* microcosms compared to those from *Gf* ones. Isolated earthworms (*If* and *Il*) showed significantly higher rates than those cultured in groups of three (*Gf* and *Gl*), as did laboratory-reared individuals (*Il* and *Gl*) when compared with field-collected earthworms (*If* and *Gf*) (Table 1).

Aestivation was observed in a variable percentage of field-collected individuals during the experiment in both types of microcosm (*If* and *Gf*), generally after the summer period. The percentage of individuals in aestivation varied between 5% and 25% of the total clitellate individuals in each type of microcosm. As this percentage was similar in both types of microcosm, mean rate of cocoon production in aestivation periods was compared, but showed no significant differences ($F_{(1,19)}=1.46$, $P=0.239$). This process was only observed once in two laboratory-reared individuals.

Discussion

A. trapezoides seems to be a suitable species for laboratory culture with its high percentage of adult and hatchling survival, and high cocoon viability. Taking into account the fact that each individual could potentially produce more than 150 live hatchlings per year, this species is appropriate for laboratory experiments. Thus, the use of this species is highly recommended in applied studies, such as ecological restoration of roadside slopes (Trigo, D., unpublished data), biological agriculture or ecotoxicological tests, as suggested by Lowe and Butt (2007) for parthenogenetic earthworms.

The rates of cocoon production and the number of hatchlings per cocoon vary enormously among earthworm species. The rate recorded in this study for *A. trapezoides* (105 cocoons per earthworm per year) is higher than the rate recorded in other anecic or endogeic species, such as *Octodrilus complanatus* (52 cocoons per earthworm per year; Monroy et al., 2007), or *Lumbricus terrestris* (38 cocoons per earthworm per year; Butt, 1993). This rate places this species closer to epigeic species such as *Eisenia fetida* (70 cocoons during its 27 weeks of life; Hartenstein et al., 1979).

The results of this laboratory study can be compared with those of McCredie et al. (1992) who studied the population dynamics of *A. trapezoides* under field conditions in Australia. Several traits such as number of hatchlings per cocoon, incubation time and weight after reaching maturity were similar in both studies. Due to the short experimental time in the McCredie et al. field experiment, nothing was revealed about the other traits investigated in the present study, which provide additional information for a complete picture of the life cycle of this important species. Although results are quite similar under both field and laboratory conditions, more data about the life cycle of this species in the field will be useful for comparisons of other life traits, such as cocoon viability, length of life or cocoon production.

In contrast to the results obtained by Lowe and Butt (2008) for *Octolasion cyaneum*, growth rates and fecundity of *A. trapezoides* were not influenced by the individual status of singletons or twins, although hatchling singletons had a mean weight almost twice that of twins. After the first 15 days, weight was not significantly different between both types of individuals, but mortality rates were higher in twins during this initial period. Lowe and Butt (2008) suggested that twins are likely to survive under adverse environmental conditions. The addition of twins would provide an advantageous increase in the population during the colonisation of new areas, when the number of individuals would outweigh the advantage of individual size. In *A. trapezoides* however, things seem to be different: twin/singleton status does not have an influence on individual size, but does influence mortality rate during the first 15 days of laboratory life. Thus, a trade-off seems to exist between the number of hatchlings per cocoon and mortality rates during the first days.

For field-collected individuals, mean rates of cocoon production were statistically similar to isolated earthworms and to those cultured in groups of three. Thus, the presence of other individuals in the same microcosms has neither a positive (for example, enhancing reproduction) nor a negative effect on this rate. Nevertheless, it is higher in laboratory-reared isolated individuals than those in groups of three. Density of earthworms influences growth, weight and fecundity of adults. For example, reproductive capacity of *L. terrestris* was reduced when cultured at high densities (15–25 g of earthworms per 2 l of soil; Lowe and Butt, 2005). In this study, it is suggested that three earthworms (a total weight of 4–6 g) could be considered a high density for *A. trapezoides*, hence producing a negative effect on potential cocoon production (which has only been recorded in laboratory-reared individuals) among individuals in the same microcosms. It can be hypothesised that in microcosms with field-collected individuals this physical disturbance was lower and had no significant impact due to the periodical inactivity of the earthworms.

All individuals of *A. trapezoides* kept in isolation produced viable cocoons during the whole experimental period, hence completely ruling out both the existence of pseudogamy and the necessity of physical–chemical stimulus to trigger reproduction in this species. Cocoon production was higher in isolated earthworms (taking into account both field-collected and laboratory-reared ones) than in those cultured in groups of three. As some field-collected individuals entered aestivation several times during the experiment, full reproductive capacity was not shown. In contrast, laboratory-reared earthworms showed their potential capacity, as they rarely entered aestivation. It is worth mentioning that cocoon production was recorded during the first year of life in laboratory-reared individuals, but the age of field-collected earthworms could not be determined. Reproduction has been shown to be age-dependent in some invertebrates, but only a few examples can be found in the literature (e.g., in the cercariae *Transversotrema patialense*; Mills, 2002). It might be of interest to test whether cocoon production varies with the age of *A. trapezoides.*

In earthworms, both sexual and parthenogenetic reproduction is observed, thus making this group suitable for studying models of animal reproduction. Although there is much scientific literature on the subject, no explanation has been universally accepted about the origin and maintenance of sexual and asexual reproduction. Asexual species have been traditionally considered as "dead end streets" that would be unavoidably extinguished due to their lower evolutionary capacity. Many questions still remain open about the biology of *A. trapezoides*. For example, does this species have a "general purpose genotype" (being able to adapt to a wide range of habitats) or is there a high clonal diversity (each clone being able to differentially adapt to a specific ecological niche)? Did it originate by hybridization from closely related species? Is its genetic diversity high or low? Despite these evolutionary questions, its biological success is unquestionable. Not only is *A. trapezoides* one of the most abundant earthworm species, but it is also very successful under different environmental conditions. Thus, both its importance as a possible key species in soil management and its highly recommended use in applied studies must be highlighted.

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References

Blakemore, R.J., 1999. The diversity of exotic earthworms in Australia – a status report. In: Ponder, W., Lunney, D. (Eds.), The Other 99%. The Conservation and Biodiversity of Invertebrates. Transactions of the Royal Zoological Society of New South Wales, Mosman, Australia, pp. 182–187.

Blakemore, R.J., 2006. Cosmopolitan Earthworms – An Eco-Taxonomic Guide to the Peregrine Species of the World, second ed. VermEcology, Japan.

Brafield, A.E., Llewellyn, M.J., 1982. Animal Energetics. Blackie and Son Ltd., Glasgow, UK.

Bouché, M.B., 1972. Lombriciens de France. Écologie et Systématique. INRA, Paris 671 pp.

Butt, K.R., 1993. Reproduction and growth of three deep-burrowing earthworms (Lumbricidae) in laboratory culture in order to assess production for soil restoration. Biol. Fert. Soils 16, 135–138.

De Mischis, C.C., 1997. Earthworms (Annelida, Oligochaeta) of a provincial reserva in Córdoba, Argentina: a preliminary survey. Soil. Biol. Biochem. 29, 235–236.

Eriksen-Hamel, N.S., Whalen, J.K., 2006. Growth rates of Aporrectodea caliginosa (Oligochaetae: Lumbricidae) as influenced by soil temperature and moisture in disturbed and undisturbed soil columns. Pedobiologia 50, 207–215.

Fraser, P.M., Williams, P.H., Haynes, R.J., 1996. Earthworm species, population size and biomass under different cropping systems across the Canterbury Plains, New Zealand. Appl. Soil Ecol. 3, 49–57.

Gates, G.E., 1972. Contributions to North American earthworms (Annelida: Oligochaeta). No. 3. Toward a revision of the earthworm family Lumbricidae. IV. The trapezoides species group. Bull. Tall Timbers Res. Stn. 12, 1–146.

Hartenstein, R., Neuhauser, E.F., Kaplan, D.L., 1979. Reproductive potential of the earthworm *Eisenia foetida*. Oecologia 43, 329–340.

Jaenicke, J., Selander, K., 1979. Evolution and ecology of parthenogenesis in earthworms. Am. Zool. 19, 729–737.

Karmegan, N., Daniel, T., 2009. Growth, reproductive biology and life cycle of the vermicomposting earthworm, *Perionyx ceylanensis* Mich. (Oligochaeta: Megascolecidae). Bioresource Technol. 100, 4790–4796.

Keselman, H.J., Algina, J., Kowalchuk, R.K., 2001. The analysis of repeated measures designs: a review. Br. J. Math. Statist. Psychol. 54, 1–20.

Lowe, C.N., Butt, K.R., 1999. Interspecific interactions between earthworms: potential applications in soil amelioration. Pedobiologia 43, 808–817.

Lowe, C.N., Butt, K.R., 2005. Culture techniques for soil dwelling earthworms: a review. Pedobiologia 49, 401–413.

Lowe, C.N., Butt, K.R., 2007. Earthworm culture, maintenance and species selection in chronic ecotoxicological studies: a critical review. Eur. J. Soil Biol. 43, 281–288.

Lowe, C.N., Butt, K.R., 2008. Life cycle traits of the parthenogenetic earthworm *Octolasion cyaneum* (Savigny, 1826). Eur. J. Soil Biol. 44, 541–544.

McCredie, T.A., Parker, C.A., Abbott, I., 1992. Population dynamics of the earthworm *Aporrectodea trapezoides* (Annelida: Lumbricidae) in a Western Australian pasture soil. Biol. Fert. Soils 12, 285–289.

Mills, C.A., 2002. Age- and density-dependent growth within populations of the ectoparasitic digenean *Transversotrema patialense* on the fish host. Int. J. Parasitol. 10, 287–291.

Monroy, F., Aira, M., Gago, J.A., Domínguez, J., 2007. Life cycle of the earthworm *Octodrilus complanatus* (Oligochaeta, Lumbricidae). C. R. Biologies 330, 389–391.

Muldal, S., 1952. The chromosomes of the earthworms. I. The evolution of polyploidy. Heredity 6, 55–76.

Pertrusewicz, K., Macfayden, A., 1970. Productivity of Terrestrial Animals. Principles and Methods. Blackwell, Oxford.

Reinecke, A.J., Ljungstrom, P.O., 1969. An ecological study of the earthworms from the banks of the Mooi River in Potchefstroom, South Africa. Pedobiologia 9, 106–111.

Zaitlin, B., Hayashi, M., Clapperton, J., 2007. Distribution of northern pocket gopher burrows, and effects on earthworms and infiltration in a prairie landscape in Alberta, Canada. Appl. Soil Ecol. 37, 88–94.