Ökologie und Paarungsverhalten des Regenwurms *Lumbricus terrestris* in Zusammenhang mit Parasitenbefall durch *Monocystis* sp.

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I. OVERVIEW

SUMMARY OF DISSERTATION

The annelid *Lumbricus terrestris* is probably one of the best studied earthworms and serves as a model species in immunology and eco-toxicology. However, little attention has been paid on the influence of parasite infections on reproduction in this species. In my research project, I focus on the effects of infection with *Monocystis* sp. (Gregarinidae, Apicomplexa, Protozoa) on fitness parameters, mating behaviour and assortative mating in laboratory and natural populations.

OUTLINE OF THE THESIS

This thesis is organised in four chapters. Chapter I is a general introduction with a literature review, while Chapter II-IV contain detailed information on three separate experiments with introduction, materials and methods, results and discussion. Chapter I provides an overview about sexual selection and the role of parasites therein. It shows the variety of hermaphroditic systems in the animal kingdom and points out how sexual selection could work in hermaphrodites. At the end, details on the study system, *Lumbricus terrestris* and *Monocystis* are presented. In Chapter II, I ask whether *Monocystis* infection influences mate choice in *L. terrestris*. Uninfected control animals are virtually absent in natural populations, so I used hand-reared, parasite-free animals. These were exposed to a new experimental infection technique followed by a test for mating latency when paired to a focal partner. Chapter III covers the question which traits play a role in earthworm mate choice and whether similarity to oneself is one of them. For this purpose, earthworms were kept in groups of six under long-term video surveillance. In Chapter IV, I describe the results of a sampling study in natural populations under different agricultural regimes. The objective was to examine effects of organic and no tillage farming on the general constitution of *L. terrestris* and the underlying genetics of both host and its parasite *Monocystis sp*.

ZUSAMMENFASSUNG DEUTSCH

Kapitel I ist eine allgemeine Einleitung mit einer Literaturübersicht, während Kapitel II-IV detaillierte Informationen über drei separate Experimente mit Einleitung, Material und Methoden, Ergebniss und Diskussion enthalten.
Kapitel I gibt einen Überblick über sexuelle Selektion und die Rolle, die Parasiten dabei spielen. Es zeigt die Vielfalt hermaphroditischer Systeme im Tierreich und erklärt, wie sexuelle Selektion bei Hermaphroditen funktioniert. An Ende werden detaillierte Informationen über das verwendete Modellsystem, *Lumbricus terrestris* und *Monocystis sp.* angegeben.

In **Kapitel II** behandele ich die Frage, ob *Monocystis* Infektionen die Partnerwahl bei *L. terrestris* beeinflussen. Uninfizierte Kontrolltiere sind fast niemals in Freilandpopulationen zu finden, deswegen benutzte ich parasitenfreie Laboraufzuchten. Diese wurden mittels einer neuen experimentellen Infektionstechnik infiziert, um dann die Paarungsfreudigkeit mit uninfizierten Tieren zu messen. Ich fand heraus dass Regenwürmer mehr Baubesuche machen, wenn sie mit einem infizierten Partner konfrontiert waren, und dass diese Baubesuche auch länger andauern. Außerdem führten höhere Parasitenkonzentrationen bei den infizierten Partnern dazu, dass diese Pärchen länger zögern, sich zum ersten Mal zu paaren. Ich schließe daraus, dass Partnerwahl im Zusammenhang mit Parasitenlast **vor** der Paarung eine wichtige Rolle für *L. terrestris* spielt.

Kapitel III befasst sich mit der Frage, welche Eigenschaften eine Rolle für die Partnerwahl bei Regenwürmern spielen und ob Ähnlichkeiten zwischen den Partnern eine solche Eigenschaft ist. Zu diesem Zweck wurden Regenwürmer in 6er Gruppen unter Langzeit-Videoüberwachung gehalten. Ich konnte zeigen dass Parasiteninfektionen mit einer niedrigeren Spermienkonzentration, schlechterem Wachstum und einer weniger deutlichen Färbung zusammenhängen. Die Bildung von zueinander passenden Paaren geschah im Zusammenhang mit der Anzahl der gebildeten Kokons, mit der Färbung und mit der Spermienanzahl, sowohl einzeln als auch in Kombination dieser Parameter. Allgemein vermieden es die Regenwürmer, sich über längere Strecken zu verpaaren, daher korrigierte ich

meine Analyse für diesen Effekt. Ich beschreibe außerdem ein neues Verhalten, dass einzelne Individuen gegenüber kopulierenden Paaren zeigen.

In **Kapitel VI** beschreibe ich die Ergebnisse einer Freilandstudie von Regenwürmern, die unter unterschiedlichen Agrarbedingungen leben. Das Ziel war, die Auswirkungen von biologischer Landwirtschaft und Direktsaatverfahren auf die allgemeine Konstitution und die zugrundeliegende Genetik sowohl vom Wirt als auch vom Parasiten zu untersuchen. Die Demographie unterschied sich zwischen den Feldtypen insofern als dass die Würmer von Biofeldern älter sind und oft einen verkürzten Körper haben, wohl eine Konsequenz aus den Verletzungen durch den Pflug. Die Infektionsmuster unterschieden sich nicht zwischen den Feldtypen, aber ich konnte zeigen, dass ein Mikrosatelliten-Allele und die Anzahl von *Monocystis* Stämmen zusammenhängen. Die Ergebnisse dieser Studie bilden eine wichtige Grundlage für Praxis-Empfehlungen an Landwirte, die eine Umstellung auf das Direktsaatverfahren anstreben.

II. GENERAL INTRODUCTION

SEXUAL SELECTION AND PARASITISM

Definition of mate choice

When Darwin first described the theory of “Mate Choice”, he was looking for an evolutionary mechanism to account for elaborated ornaments that would otherwise remain unexplained by natural selection. “Mate Choice” was defined as an intersexual selection by which individuals of one sex evolve traits that are *preferred* by members of the opposite sex (Darwin 1871). In today´s broader sense definition, the occurrence of non-random mating in one sex regarding one or several varying traits of the other sex is called mate choice (reviewed in Bradbury, Anderson et al. 1987; Leonard 2006).

Anisogamy as primary source for sexual selection

It is assumed that at the evolutionary origin of sexuality, ancestral organisms were isogamous. The subsequent divergence into two sexes, one with many, small gametes (males), and one with few, large gametes (females) and associated differential investment into offspring is seen as a kind of “male parasitism” by some authors (Hosken 2009; Parker and Pizzari 2010; Lehtonen and Kokko 2011). However, opposing opinions advocate a cooperative rather than exploitative scenario in recent publications (Iyer and Roughgarden 2008; Yang 2010). The fact that most organisms are anisogamous has nevertheless important consequences for the evolutionary paths of the different sexes (Parker, Baker et al. 1972; Clutton-Brock and Parker 1992; Kokko and Monaghan 2001; Bulmer and Parker 2002).

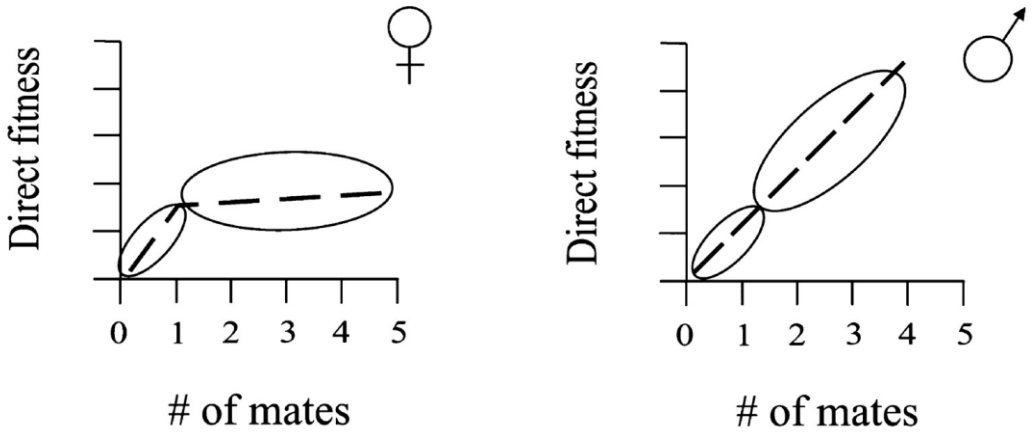

Figure 1 Example for the relationship between number of mates and direct fitness in females and males.

Whereas female fitness is limited by the resources necessary for ova production, male fitness is primarily limited by access to mating partners and fertilization opportunities. Thus, the relationship between the number of copulations and resulting reproductive output differs between the sexes such that male reproductive output accumulates more and longer with additional copulations, than it does in females (**Figure 1**). Since all males can only mate as often as all females do, males will compete with other males for females. They have an advantage from mating with many different partners and are generally more eager to mate. On the contrary, females should choose their partners carefully (Cunningham and Birkhead 1998; Hoffer, Ellers et al. 2010) because they can only optimize their reproductive success through the quality of their offspring. Males not only provide genetic material to insure this high quality of offspring, but could also provide additional resources such as nuptial gifts or assistance in raising young.

Mechanisms and levels of sexual selection

Females can promote their fitness by choosing mates who provide immediate, direct benefits, e.g. through nuptial gifts, parental care or reduced parasite transmission (Andersson 1994). In addition, the existence of a genetic basis for indirect fitness gains through mate choice have been widely discussed (Kirkpatrick and Ryan 1991; Charmantier and Sheldon 2006; Kokko, Jennions et al. 2006). For example, females could also look for “good genes” in potential fathers to increase their own breeding value, e.g. through enhanced

parasite resistance in their young (Milinski and Bakker 1990; Møller 1992). Surviving offspring will pass on the genes for both the favourable male trait and the female taste for it. This positive feedback loop between male attractiveness, and female preferences beyond a naturally selected optimum or for arbitrary traits has come to be known as the “Fisherian Runaway” process (Fisher 1930). In order to start such a substantial genetic correlation between male attractiveness and female preference, males might exploit a female trait already selected for by natural selection. Female sensory bias towards a certain food colour or predation avoid have been reported to be astonishingly similar to their preferred male trait (Endler and Basolo 1998; Sakaluk 2000). A female might even try to find a mate that will sire attractive sons although this poses an immediate cost on her overall reproductive success (sexy son hypothesis, Weatherhead and Robertson 1979). When taking into account on how these “sexy sons” perform during their lifetime, it is actually applicable to see processes such as Fisherian Runaways and acquisition of “good genes” through sexy sons as equal pathways to the same outcome (Kokko 2001). In contrast to the abovementioned attractiveness principles, the handicap hypothesis of (Amotz 1975) states that over-elaborated, maladaptive sexual traits prove overall health or physical strength to the choosing female (Wedekind 1992)

It is now increasingly recognized that simple scenarios such as choice for inbreeding avoidance (Amos, Wilmer et al. 2001; Tregenza and Wedell 2002; Lehmann, Keller et al. 2007) or choice for additive genetic variation (i.e. the lek paradox, Kirkpatrick and Ryan 1991; Blows, Chenoweth et al. 2004; Tomkins, Radwan et al. 2004) describe small components of the bigger picture - female choice is likely to be e.g. genotype or condition dependent (Mays Jr and Hill 2004; Neff and Pitcher 2005). Critical mate assessment is not the only barrier males need to pass before insemination (Pizzari and Snook 2003): In natural situations, females may be reluctant to mate per se, and males need to overcome female defenses especially in costly, enforced copulations (Thornhill 1980). Nevertheless, female remating behaviour often exceeds their mating rate optimum (Arnqvist, Nilsson et al. 2005). Mating with multiple partners (polyandry) must therefore be advantageous and outweigh

immediate mating costs (Keller and Reeve 1995; Arnqvist and Nilsson 2000). Parker (1970) first proposed for internally fertilizing species that female choice might continue **after** copulation, and female control over fertilization might well represent female mate choice (Eberhard 1996; Gowaty 2004) This cryptic female choice can be expressed in female behaviour, physiology and genital morphology such as selective use of sperm to fertilize the ova (Thornhill 1983; Eberhard 1991; Eberhard and Cordero 1995) and sperm competition (Keller and Reeve 1995) , but may continue in post-zygotic stages (Wedekind 1994) by selective abortion (Kozlowski and Stearns 1989), neglect or cannibalism (Elgar 1992).

Host-parasite coevolution and sexual reproduction

Parasites continuously evolve new mechanisms to infect and exploit hosts, while hosts adapt their resistance to the new parasite adaptations (i.e. the Red Queen Hypothesis, Van Valen 1977). Frequency-dependent selection of parasite virulence and host defence strategies can lead to cycles of coevolution (Lohse, Gutierrez et al. 2006; Ebert 2008; Poullain, Gandon et al. 2008) in which heritable genetic variation in both host and parasite are generated over and over again (Eshel and Hamilton 1984; Lively and Dybdahl 2000; Decaestecker, Gaba et al. 2007). In order to quickly produce genetically variable offspring, sexual reproduction with constant mixture of parental genomes is advantageous (Bell 1982; Hamilton 1990; Crow 1994) and leads to accelerated adaptation (Lorch, Proulx et al. 2003). To make sexual reproduction even more successful, sexually dimorphic species can improve offspring resistance through mate choice based on secondary sexual characters (ornaments) that truly display the parasite infection status of its carrier (Hamilton and Zuk 1982; Kennedy, Endler et al. 1987; Møller 1990; Wedekind 1992). For a more detailed review on the influential Hamilton and Zuk hypothesis see Chapter III.

HERMAPHRODITISM AND MATE CHOICE

Evolution and varieties of hermaphroditism

Charnov first stated in his "sex-allocation theory" that being hermaphroditic and possessing both male and female genitalia is favourable when the combined reproductive output of both functions exceeds those of being only male or female (Charnov, Bull et al. 1976; Charnov 1982). Hermaphroditism is widespread throughout the plant and animal kingdom, where it is predominantly described in invertebrates (excluding insects) and fish (see detailed review scheme in (Anthes 2010)). In fish, size-dependent sex change (i.e. sequential hermaphroditism) is frequent due to differences in the size-fecundity relationship (Warner 1975; Munday, Buston et al. 2006), whereas simultaneous hermaphrodites express both sexes at the same time and consequently have the opportunity to either self-fertilize or outcross (Kleemann and Basolo 2007; Koene, Montagne-Wajer et al. 2007). I here focus on exclusively outcrossing hermaphroditism and its multifaceted consequences on sexual selection.

Iyer and Roughgarden (2008) inferred from phylogenetic analysis that simultaneous hermaphroditism is the ancestral state for animals, possibly because it is advantageous to have both sexual functions when mate encounter is rare, e.g. in populations with low density (Charnov, Bull et al. 1976), highly structured populations or immobile animals (Charnov 1987). Furthermore, simultaneous hermaphrodites can adjust the allocation of resources to either sexual function more flexible – not only within their life time (Angeloni, Bradbury et al. 2002; Brauer, Schärer et al. 2007) but even between reproductive events (Loose and Koene 2008).

Mate choice in hermaphrodites

Despite this flexibility, most individuals of a hermaphroditic species are quite similar to each other and share the same interests during copulation: Because sperm is cheaper to produce and paternal fitness much easier to achieve, hermaphrodites are expected to prefer the role of the sperm donor. This can lead to conflicts on who is allowed to inseminate the partner and results in

strong sexual antagonistic coevolution. For sure, mechanisms such as Fisherian runaways and "sexy sons" function differently or slower in hermaphrodites because all sexually relevant genes are expressed in every generation rather than e.g. female traits staying suppressed in males (Michiels and Newman 1998). For a detailed review on mate choice mechanisms in hermaphrodites, see Chapter III.

THE SYSTEM UNDER INVESTIGATION

Lumbricus terrestris

Figure 2 Adult *Lumbricus terrestris (Foto S.Weller)*

The earthworm *Lumbricus terrestris* (Annelida, Clitellata, Oligochaeta) (**Figure 2**) was first described by Linnaeus in 1758. Also known as the Common/Canadian Nightcrawler or dew worm, it is one of the most intensely studied species throughout history. When Darwin published his book "On the formation of mould through the action of worms" in 1881, he feared that "the subject of it will not attract the public", but the book was sold out within days. Since then, earthworm research has focused mainly on its impact on soil structure (James, Eckert et al. 2005) and productivity (Gibson 1996), its use in ecotoxicological tests (Rodriguez-Castellanos and Sanchez-

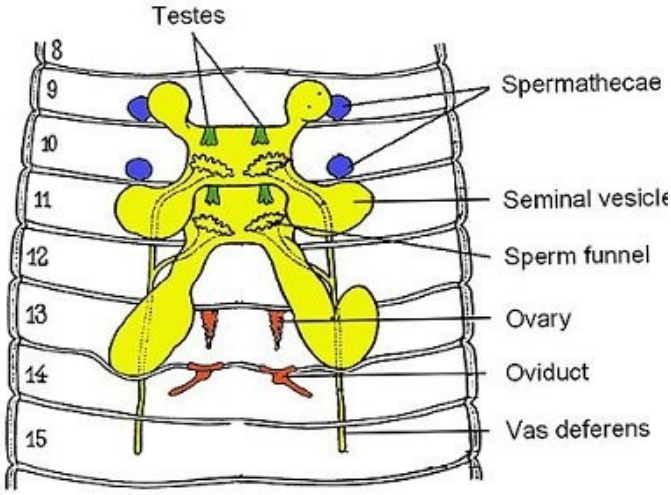

Figure 3 Schematic view of sexual organs from dorsal (adapted from Storch & Welsch (1996))

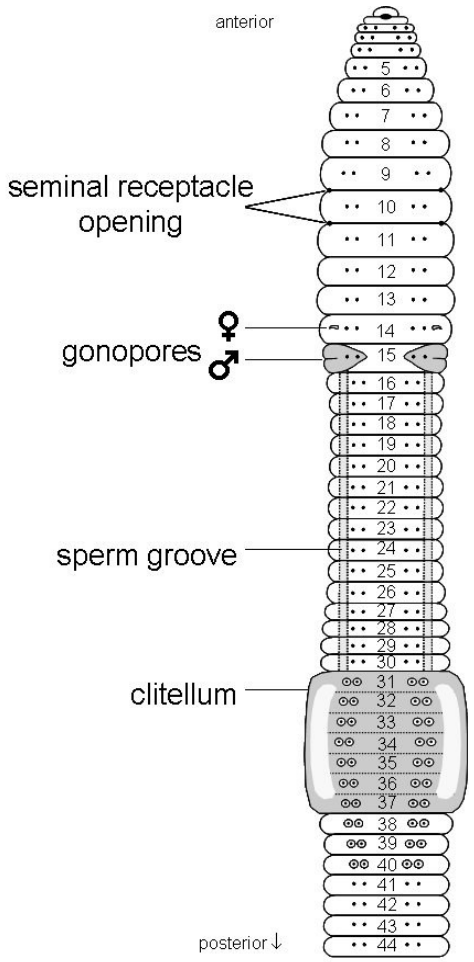

Figure 4 Schematic ventral view, adapted from N. Michiels

Hernandez 2007) and immunoassays (Cooper and Roch 2003). Earthworms are coelomate protostomes with a tube-like, segmented body structure (**Figure 2**). The inner tube forms the gut with a buccal cavity, pharynx, oesophagus, crop, gizzard and anus. The space between the digestive system and the outer muscular tube includes a closed blood circulatory system with haemoglobin in free suspension, an organized nervous system, metanephridia for excretion and five aortic arches. The reproductive organs are located in the front part. As an obligatory outcrossing hermaphrodite, *Lumbricus terrestris* possesses male and female organs with ventral pores (**Figure 4)**. Between segments nine and ten are two pairs of seminal receptacle pores that allow the entrance of allosperm into the seminal receptacles or spermathecae. These are pinhead-like pouches that can store sperm for several months. At segment 14, the female gonopores release oocytes from the ovaries during the cocoon building process. The male gonopores are located at segment 15 and can release sperm from the testis into two sperm grooves. These run left and right of the ventrum towards the clitellum (Segment 31-37), a special girdle used during copulation and cocoon production. Note that although sperm cells are produced by the small testes, but they mature in six large seminal vesicles that fill most of the anterior body cavity (**Figure 3**). The epidermis of the anterior region is pigmented in dark red-brownish colours that slowly fade into a light pink towards the tail end (**Figure 2**). Along with this colouration pattern, a unique characteristic of

Lumbricus terrestris is its ability to flatten the tail end, resulting in a tight anchoring in its burrow.

The formation of vertical, deep (~ 1 m) burrows with only one surface opening is one of the main characteristics of *Lumbricus terrestris´* life style. Individuals stay in their burrows with the tail end while searching for food on the surface. When they reach dead plant material, it is pulled into the burrow for further decay before consumption. Faeces are often deposited above ground at the burrow entrance and form typical earthworm casts. In contrast to other epigeic (litter-dwelling) or endogeic (deep soil-dwelling) earthworms, the anecic, semi-sessile life style together with a relatively slow reproduction rate of *Lumbricus terrestris* could lead to low dispersal rates of approximately 4m/year (Warner 1995). Although occurring in quite high densities in undisturbed habitats (e.g. 62 individuals/m^2, Krupp, Kent et al. 2008), they are also limited within their surface action radius of about 30 cm around the burrow opening when searching for potential mating partners.

An important reproductive feature of *Lumbricus terrestris* is its habit to mate on the soil surface, which allows for direct or video observation. The general reproductive mechanisms of earthworms are well understood, it is important to mention that copulation and cocoon production are two separate processes. Earthworms reach sexual maturity when they have a fully developed clitellum. In *Lumbricus terrestris*, this takes, depending on climatic and feeding conditions, around 6 months. The mating sequence is initialised by close body contact at the first segments, often followed by one to several, often reciprocal “burrow visits”. The partners stick their head into the other earthworm´s burrow, which stimulates the resident to emerge from its burrow and reciprocate the visit. This “courtship” behaviour can go on for hours and does not always result in copulation in the same night, but increases the likelihood of copulation in later nights. The actual copulation starts when both partners slide along their ventral sides until segments 9-11 with the sperm receptacle pores reach the partner’s clitellum.

Figure 5 Copulation of *Lumbricus terrestris (*© Premafotos Wildlife)

Partner contact is tightened by insertion of special copulatory setae (segment 10, 26, 31-38) into the partner and results in a typical S-shape position of the pair (**Figure 5**, Koene, Sundermann et al. 2002), while the tail ends stay in their respective burrows (Barnes et al. 1993). Sperm is then transferred along the ventral sperm grooves to the seminal receptacles of the partner. The transfer lasts between 20 min and 3 h (personal observations) and pairs are less prone to disturbance than individuals. The mating sequence is finished by a rapid disruption of the S-shape. The partners immediately retreat below ground. Shortly after copulation they start with normal feeding behaviour again, usually without any sign of cocoon production. Cocoon production takes place below ground and cocoons are deposited at depth between 0.4 and 0.05 m (Butt 2002). They are only produced after at least one copulation, but allosperm can be stored up to six months (Butt 1993). During cocoon production, the clitellum secretes a ring structure that hardens when in contact with air. The earthworm slowly crawls backwards, sliding the ring over its anterior region. This “ring” is then moved forward by peristaltic body contractions. While passing the female gonopores and the sperm receptacle pores, one or more oocytes and allosperm are deposited in the developing cocoon, resulting in fertilization. Once the ring-shaped cocoon has past the head, both openings close spontaneously.

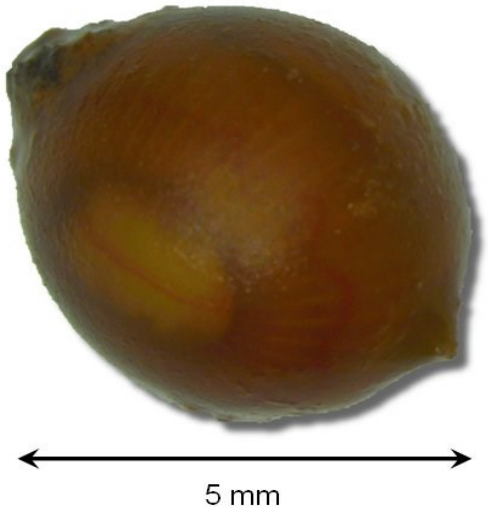

Figure 6 *Lumbricus terrestris* cocoon. Note the blood vessels and the segmented body structure of the embryo. This picture was taken shortly before hatching, and regular movement within the cocoon could be observed.

Cocoons are first white and soft and then harden within minutes (S. Weller, personal observation) to a dark golden or brown spherical structure with pointed ends (**Figure 6**).

Monocystis

In European populations, up to 99% of *L. terrestris* individuals are infected with the protozoan parasite *Monocystis* sp. (Sahm, Velavan et al. 2009) *Monocystis* is a basal representative of the Apicomplexa group and belongs to the Gregarines (Leander, Clopton et al. 2003). Only a few experts can identify *Monocystis* species based on morphological characters of the (rarely isolated) trophozoid stage (Berlin 1923).

Worms become infected when they ingest *Monocystis* oocysts, also called sporocysts or simply spores, each of which contains several sporozoites. These hatch in the gizzard, penetrate the intestinal wall, enter a dorsal blood vessel, and move forward to the hearts. They then leave the circulatory system and penetrate the seminal vesicles, where they enter the sperm-forming cells (blastophores) in the vesicle wall. After a short period of growth during which they probably destroy developing host spermatocytes, sporozoites enter the vesicle lumen where they mature into trophozoites, or gamonts. Gamonts attach to cells near the sperm tunnel where they undergo syzygy, in which two or more gamonts connect with one another in tandem. Gamont pairs then surround themselves with a common cyst envelope,

forming a gametocyst. Afterwards, each gamont undergoes numerous nuclear divisions to form many small gametes. Gametes fuse to form a zygote and secrete an oocyst membrane around that zygote. The membrane around the oocyst will later harden to give spores the typical fusiform shape (**Figure 7**). Only zygotes are diploid, and subsequent meiosis returns the cells to the haploid stage. Two or three cell divisions follow to form eight sporozoites inside one spore. Gametocysts or, if it ruptures, the contained spores, pass out through the sperm duct to be ingested by another worm. It is known that spores survive the passing through the intestine of earthworm predators such like shrews and raccoons (Schmidt and Roberts 2005).

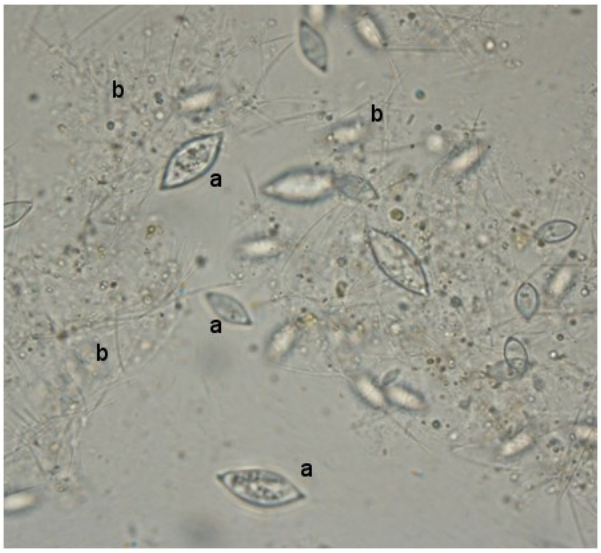

Figure 7 Smear of fresh seminal vesicle
a *Monocystis* sporocysts, b Autosperm clumbs

The application of new ribosomal DNA markers revealed that *Monocystis* is genetically highly variable with more than one strain in a single host individual being the rule (Velavan, Schulenburg et al. 2010). Otherwise, the deleterious effects of *Monocystis* are subtle, mainly affecting earthworm growth. No effect could be detected on copulation rate and activity (Field and Michiels 2005). Some authors claim that heavy infections can sometimes castrate the host (Breidenbach 2002).

OBJECTIVES OF THIS THESIS

The earthworm *L. terrestris* has been extensively studied under the lights of immunology and ecology. However, little attention has been paid on its reproduction and parasite host interactions. Only in the 1990ies, Dr. Kevin Butt and Dr. Visa Nuutinen started to examine the importance of reproductive cycles for soil restoration and earthworm behavioural ecology (Butt 1993; Nuutinen and Butt 1997; Butt, Frederickson et al. 1999). Studies on mate choice in this simultaneous hermaphrodite have been conducted by Dr. Nico Michiels; Dr: Stuart Field and Dr. Joris Koene at the University of Münster. Their work not only paved the way for experiments with higher sample sizes, but also gave insights into the specialities of mate choice in hermaphrodites (Michiels, Hohner et al. 2001; Koene, Sundermann et al. 2002; Field, Schirp et al. 2003; Field, Kurtz et al. 2004; Field and Michiels 2005; Koene, Pförtner et al. 2005; Field and Michiels 2006; Field and Michiels 2006). Dr. T.P. Velavan established microsatellite markers that can now be used for paternity analysis and population genetics. He also developed mitochondrial markers to distinguish between different strains of the parasite *Monocystis* (Velavan, Schulenburg et al. 2007; Velavan, Schulenburg et al. 2010). The life cycle of this parasite was studied by Miles in 1962 with controlled infection experiments on a small sample set. Dr. Stuart Field managed to raise uninfected earthworms, the basis for controlled infection and mating experiments.

This dissertation should be seen as the continuation of the abovementioned work and the application and optimisation of the established methods. My studies focus on the effects of *Monocystis* infections on *L. terrestris* mating behaviour. In the first study (Chapter III), I pose the question whether *L. terrestris* chooses its mates according to the partner´s infection status and if it uses the coloration of the epidermis to do so. I use hand-raised, uninfected earthworms and controlled infections to set up a no choice experiment. Earthworms are kept in pairs with an uninfected focal individual paired either with an infected or an uninfected (control) partner, and time-lapse video observation is used to observe mating behaviour.

The second study aims to answer the question whether earthworms mate assortatively when kept in groups. I use a correlative approach on long-term video observation data of earthworm neighbourhoods with six individuals. Additionally, I record and describe earthworm group behaviour.

In the third study, I am interested in genetic and phenotypic effects of *Monocystis* on *L. terrestris* in natural populations. Earthworms are sampled from organic and no tillage fields and examined in detail for differences in phenotypic and genetic parameters. The newly developed mitochondrial markers for *Monocystis* are used to investigate differences in infection patterns.

III. PARASITE-MEDIATED PRECOPULATORY MATE CHOICE IN A SIMULTANEOUS HERMAPHRODITE

ABSTRACT

The concept of parasite-mediated mate choice makes clear predictions for separate sex species such that females should be choose their mates based on male clues that indicate parasite resistance. For hermaphrodites however, such choice scenarios can be much more complicated. Here, I investigate parasite-mediated mate choice in a simultaneous hermaphrodite with mutual sperm transfer. The earthworm *L. terrestris* performs burrow visits, often interpreted as courtship behaviour, before mating. Its sperm storage organs are usually parasitized by the protozoan *Monocystis* (sp.), which occasionally leads to castration. I expect mate choice because I used a no choice experiment with uninfected earthworms either paired to an infected or an uninfected (control) partner to address the question if parasite-mediated mate choice exists in this system, and spectral measurement of earthworm skin to reveal if skin colour could be used as clue for parasitism. I found that earthworms paired with infected partners performed more burrow visits and that these burrow visits lasted longer. Furthermore, higher parasite concentrations of infected partners lead to longer delays until the first mating started. I also show that skin colour has little, if any effect on mating behaviour. I conclude that precopulatory parasite-mediated mate choice plays an important role in *L. terrestris* and discuss my findings in the light of hermaphrodite sexual selection theory.

INTRODUCTION

Parasite-mediated sexual selection

Considering that around 40 % of animals are parasites (Dobson, Lafferty et al. 2008), one can assume that most free-living organisms will have to face the threat of parasitism. Thus, parasites have a strong influence on host life history traits, including mating decisions and copulatory behaviour (Poulin and Vickery 1996). Freeland (1976) first proposed that parasitic infections are a driving force in the evolution of mate choice. Shortly after, Hamilton and Zuk (1982) formulated their influential parasite-mediated mate choice theory: Parasite resistance, indicated by the display of honest secondary sexual traits should be favoured by the gender with higher investment into offspring. As females only have certain number of large ova and are therefore limited in their reproductive output (Bateman 1948), they mostly take the choosing role. For convenience, I will use females as the choosing gender until further notice.

Females may choose unparasitized males to avoid contagion (transmission avoidance hypothesis, Borgia and Collis 1989; Clayton 1991) or to assure that fathers are able to contribute to rearing offspring (resource provisioning hypothesis, Zeh and Smith 1985; Wedell 1991; Møller and Saino 1994). In vertebrates, males producing costly ornaments often need to suppress their immune system (via testosterone), leading to a trade-off between immunity and the quality of sexual signalling (Folstad and Karter 1992) and a similar trade-off seems to exist in invertebrates (Simmons and Roberts 2005). This so called immunocompetence handicap hypothesis states that only high quality males can afford immune suppression and should therefore be favoured by females (reviewed in Jacobs and Zuk 2011).

In contrast to the aforementioned hypotheses, the classical Hamilton and Zuk hypothesis (1982) assumes heritable host resistance. Passing on resistance genes from brightly ornamented fathers to offspring has important consequences for host-parasite coevolution: Parasites continuously evolve new mechanisms to deplete host resources, while hosts should adapt their resistance to the new challenge (i.e. the Red Queen Hypothesis, Van Valen

1977). Frequency-dependent selection of parasite virulence and host defence strategies lead to cycles of coevolution and maintain heritable genetic variation in both host and parasite populations (Eshel and Hamilton 1984). How genetic variation can be maintained when strong inherited female preferences for a particular ornament lead to fixation of the genes underlaying the trait in question has become known as the “lek paradox” (Taylor and Williams 1982; Kirkpatrick and Ryan 1991; Rowe and Houle 1996; Hine, Chenoweth et al. 2004; Tomkins, Radwan et al. 2004; Kotiaho, LeBas et al. 2008) – parasite-mediated sexual selection, together with Red Queen dynamics, has the potential to inhibit the reduction of genetic variation of life history, immune and sexually selected traits.

Beside choice patterns for absolute trait values, parasite-mediated mate choice and the maintenance of genetic variance can also be seen in the light of heterozygote advantage. Production of heterozygote offspring can be achieved via disassortative mating or by choosing genetically compatible partners (reviewed in Neff and Pitcher 2005; Consuegra and Garcia de Leaniz 2008, Zeh and Zeh 1996; Zeh and Zeh 1997). A widely studied variant of the compatible gene hypothesis is inbreeding avoidance: Females choose males as distinctively related to themselves as possible to create heterozygous offspring (e. g. for major histocompatibility complex genes, Wedekind, Seebeck et al. 1995; Landry, Garant et al. 2001; Tregenza and Wedell 2002, reviewed in Pusey and Wolf 1996).

However, the evolutionary scope for fine-tuned sexual selection highly depends on the species sensory system (Endler and Basolo 1998). For long, and possibly influenced by our own sensory bias, researchers concentrated on visual clues for mate choice (Hill 1990; Wedekind 1992; Collins, Hubbard et al. 1994; Lancaster, Hipsley et al. 2009). Recent work has been done on auditory (Gerhardt, Tanner et al. 2000; Verburgt, Ferreira et al. 2011) and olfactory (Iyengar, Rossini et al. 2001) mate choice parameters. Furthermore, empirical tests show a linkage between ornaments and parasite burden in many, but not all species (Clayton 1991; Kose and Møller 1999). Detection of parasite-mediated sexual selection can be difficult when multiple infections

with various parasites and different virulence patterns influence the evolution of sexually selected traits (Jacobs and Zuk 2011).

Sexual selection in hermaphrodites

Research on sexual selection has focused on organisms (plants excluded) with separate sexes (Snyder and Gowaty 2007; Brown, Laland et al. 2009; Hunt, Breuker et al. 2009) although a relevant portion of animals are hermaphroditic (Jarne and Auld 2006, see detailed review scheme in Anthes 2010). Simultaneous hermaphrodites (hereafter hermaphrodites) who unite both sexual function in one body have attained increasing interest in the study of sexual selection (Charnov 1979; Morgan 1994; Baur, Locher et al. 1998; Michiels and Newman 1998; Leonard 2006; Bedhomme, Bernasconi et al. 2009), e.g. in sex allocation (Charnov 1982; Angeloni, Bradbury et al. 2002; Brauer, Schärer et al. 2007; Loose and Koene 2008; Schärer and Janicke 2009), expression of sex roles during copulation (Charnov 1979; Anthes, Putz et al. 2006a; Anthes, Putz et al. 2006b; Sprenger, Lange et al. 2009) or selfing rates (Jarne and Auld 2006).

Darwin (Darwin 1871; Darwin 1874) was convicted that sexual selection cannot act in hermaphrodites such as snails and earthworms due to a lack of sexually dimorphic ornaments, but Charnov 1979 extended Bateman´s principle (Bateman 1948) of sex-specific linkage between and reproductive success to hermaphrodites: Because sperm is cheaper to produce and paternal fitness much easier to achieve, hermaphrodites are expected to prefer the male role. They should only let partners fertilize their ova when they, in return, can have their paternal share (Charnov 1979).

In gonochorists, generation jumps of sexually relevant genes allow for recombination without exposure to selection - a constant source of variation. In hermaphrodites however, such genes are expressed in every generation rather than e.g. female traits staying suppressed in males and are therefore constant subject to selection. Limited genetic variation can therefore slow down runaway evolution of such traits (Greeff and Michiels 1999b), but recent theoretical models (B. Kuijper, L. Schärer, and I. Pen, unpublished manuscript) state that anisogamy, Bateman´s principle and its consequences

for the evolution of distinct interests in the sexes also hold for hermaphrodites (Schärer, Rowe et al. 2012). In an empirical study on a gastropod snail, (Pélissié, Jarne et al. 2012) show that sexual selection, as predicted by anisogamy, acts basically similar in hermaphrodites and gonochorists. Implications of Bateman´s principle for hermaphrodites have also been discussed by (Leonard 2005) who concludes that gamete trading and control over fertilization is more important than resource considerations as predicted by Bateman. Nevertheless, most scientists agree now that sexual selection and mate choice are in general possible in hermaphrodites (Charnov 1979; Charlesworth and Charlesworth 1987; Arnold 1994; Morgan 1994; Leonard 2006).

Further empirical support for sexual selection acting in hermaphrodites has been gathered in studies about one of the potential driving forces of sexual selection in hermaphrodites - in the field of research on sexual conflict: Such conflicts can arise because most individuals of hermaphroditic species are quite similar to each other and share the same interests during copulation (Arnqvist and Rowe 2005). Hermaphrodites are expected to copulate only if they get to inseminate their partner in return as sperm is supposed to be cheaper to produce. Sperm trading (Leonard and Lukowiak 1991; Vreys and Michiels 1998) or conditional reciprocity (Michiels and Kuhl 2003; Pongratz and Michiels 2003; Schmitt, Anthes et al. 2007) are benign ways to solve this conflict. Other hermaphrodites manipulate their partners with allohormones to accept sperm (Koene, Sundermann et al. 2002; Koene, Pförtner et al. 2005), or even resort to harmful insemination (Anthes and Michiels 2007). Coevolution of such attack and defense mechanism could lead to acceleration and complication of harmful sexual traits (Michiels and Newman 1998; Koene and Schulenburg 2005; Beese, Beier et al. 2006; Michiels and Koene 2006; Anthes, Schulenburg et al. 2008; Bedhomme, Bernasconi et al. 2009; Anthes 2010). To overcome such invasive strategies, sperm digestion should be advantageous and is quite frequent in hermaphrodites (Charnov 1979; Greeff and Michiels 1999a).

This has ample consequences for the evolution of mate choice in hermaphrodites: First, when sperm becomes costly to produce and mating is

frequent, a sperm donor should become choosy about with whom to mate (Michiels 1998), and the choosing role might switch from female to the male role. While male mate choice does not evolve easily in gonochorists (Johnstone 1996; Kokko and Johnstone 2002; Schmeller, O'Hara et al. 2005; Bateman and Fleming 2006; Barry and Kokko 2010), mating preferences for larger body size, often assigned to the male role, might be the rule rather than the exception in hermaphrodites (Tomiyama 1996; Anthes, Putz et al. 2005, and see references next section). Secondly, sperm digestion might come handy when unwanted ejaculates are received, shifting mate choice towards a postcopulatory arena. This cryptic female choice (Thornhill 1983; Eberhard 1996; Pryke, Rollins et al. 2010) should therefore be most pronounced in reciprocally mating hermaphrodites (Vizoso, Rieger et al. 2010). Theoretically, internally fertilizing hermaphrodites are indeed expected to exhibit less intense pre-copulatory mate choice compared to gonochorists (reviewed by Arnqvist and Rowe 2005; Schärer and Janicke 2009; Janicke, Kesselring et al. 2012).

Nevertheless, hermaphrodite sexual morphology is often astonishingly complicated (Anthes, Schulenburg et al. 2008). Together with long, ritualised courtship behaviour, there is manifold scope for partner evaluation (Dillen, Jordaens et al. 2010; Minoretti, Schmera et al. 2011). Choice patterns are mainly reported to be size-based as body size is a good indicator for the potential of the female function to produce eggs (Schärer, Karlsson et al. 2001; Angeloni, Bradbury et al. 2003; Koene, Montagne-Wajer et al. 2007). Mating preferences for larger partners is quite frequent (Otsuka, Rouger et al. 1980; DeWitt 1996; Lüscher and Wedekind 2002; Chaine and Angeloni 2005), but might also be size-assortative if all individuals of the population prefer a larger partner and are neglected as too small (Vreys and Michiels 1997; Michiels, Hohner et al. 2001; Lüscher and Wedekind 2002; Angeloni 2003; Koene, Montagne-Wajer et al. 2007). Physical constrains such as mating on vertical substrates could favor mating between partners of different size, as was reported by (Jordaens, Pinceel et al. 2005) for *Succinea putris*. Other hermaphrodites may simply avoid partners that were recently mated (Haase and Karlsson 2004) or adjust ejaculate size when sperm competition is

expected (DeWitt 1996; Loose and Koene 2008; Velando, Eiroa et al. 2008). There is also empirical evidence for inbreeding avoidance and kin-discrimination (McCarthy and Sih 2008; Escobar, Auld et al. 2011). Some simultaneous hermaphrodites assume different gender roles in subsequent copulations (Leonard and Lukowiak 1991) and can thus choose their partners preferred by their current sexual function (Angeloni 2003; Sprenger, Lange et al. 2009).

Evidence for parasite-mediated sexual selection in hermaphrodites is still scarce, but Howard and Lively´s (2003) model for maintaining sexual reproduction in a putative hermaphroditic population results in an overall benefit through mate choice for resistant genes. To my knowledge, only Webster (Webster, Hoffman et al. 2003; Webster and Gower 2006) could show in a very elegant study that parasite resistant *Biomphalaria glabrata* snails refused to mate in the female role with infected partners and that these snails are capable of recognizing an infection or resistant genotype in potential partners.

Perception of parasitic infections requires sensory assessment of the partners, this can involve anything from short body contacts (Anthes 2007) to prolonged precopulatory behavior. i.e. in the flatworms *Dugesia, gonocephala* (Vreys and Michiels 1997) and *Macrostomum lignano* (Janicke, Kesselring et al. 2012). The hermaphroditic earthworm *L. terrestris* exhibits extensive precopulatory behaviour during which individuals enter into the burrows of neighbouring conspecifics with their anterior ends (Nuutinen and Butt 1997). It has been proposed that these burrow visits are used to evaluate partner body size (Michiels, Hohner et al. 2001). Size-assortative mating has been reported for another earthworm species that does not perform burrow visits (Monroy, Aira et al. 2005), and the benefits of repeated burrow visits still lack explanation.

The system under investigation

In this study, I investigate parasite-mediated mate choice in the Canadian nightcrawler *Lumbricus terrestris* (Linnaeus, Clitellata, Oligochaeta, Lumbricidae). This rather long-living and large earthworm has an anecic life

style with vertical burrows as shelters, but constant surface activity during the night. With its tail remaining in the burrow, it feeds and mates on the surface. During copulation, two individuals are tightly attached head-to-tail with their anterior parts (Nuutinen and Butt 1997), and sperm is transferred mutually through outer sperm grooves into sperm storage organs (spermathecae).

Seminal vesicles (where self-sperm is produced) of *L. terrestris* are inhabited by the protozoan parasite *Monocystis sp*. (Apicomplexa, Eugregarinoidae) (Schmidt and Roberts 2005), and heavy infections are suspected to castrate the host (Sims and Gerard 1985). Demonstrated negative effects of *Monocystis* are subtle, mainly affecting earthworm growth but not cocoon production (Field and Michiels 2005) or general activity (Field and Michiels 2006). In a correlative study using specimen from the wild, (Field, Schirp et al. 2003) could not detect any effect on copulation rate. (Field and Michiels 2006) used captive reared, uninfected *L. terrestris* to demonstrate that *Monocystis* is not transmitted during copulation and that trophical transmission is most plausible. Overall, the immediate fitness costs of *Monocystis* seem to be low, and it has been suggested that gregarine host-parasite relationships are old and therefore rather benign (Farmer 1980). Nevertheless, a recent correlative study found that high infection levels pose a cost on sperm production (Chapter IV). In a gregarine-dragonfly system, parasites had a systemic effect on muscles, thereby reducing flight display performance. As a consequence, males were considered less attractive by females (Marden and Cobb 2004) in mate choice experiments.

Additionally, I expect mate choice in *L. terrestris* because both copulation and cocoon production are rather costly when compared to other earthworm species: Predation risk during copulation is high because mating takes place on the surface and pairs remain immobile for 2-3 hours (Cosín, Novo et al. 2011). Further bonding of pairs is assured by the use of copulatory setae. They are pierced into the partner's epidermis at the clitellum and can cause substantial damage. Along with this wounding, injection of setal gland products influences sperm uptake, which may hint at potentials for sexual conflict (Koene, Pförtner et al. 2005). The male function should not be restricted by mating opportunities because *L. terrestris* often occurs in high

density (Velando, Eiroa et al. 2008), but the female function seems to be energy limited and reproduction is rather slow. Generation time is about 6 month under optimal laboratory conditions and only approximately one cocoon is produced every week, each containing a single young (twin rate 1%, Butt 1993).

Additionally, cocoons are placed near the surface (Butt 2002) and hatching young can be found in casts (Butt and Lowe 2007) and may feed on adult faeces. Later, semi-adults might settle down in the burrows of dead individual and even consume part of the cadaver containing *Monocystis* spores. Therefore, infection risk is quite high and could explain prevalences of up to 99% in natural populations (see Chapter V). Bearing in mind that *Monocystis* infections seem to have an effect on the outer coloration of the worms (Field, Schirp et al. 2003 and Chapter IV), and that earthworms possess light receptors in their head region (Hess 1925), *L. terrestris* might well be able to use indirect cues for *Monocystis* infection as mate choice criteria.

Hypothesis & Expectations

This study was designed to address the following questions posed by the *Monocystis-Lumbricus* system:

- What are the fitness costs of *Monocystis*?
- Does *L. terrestris* exhibit parasite-mediated mate choice regarding to *Monocystis* infection?
- Could skin colour be used to assess *Monocystis* infection and adjust mating behaviour accordingly?

I tested these hypotheses by combining a no-choice experiment with controlled infection. To accurately determine effects of *Monocystis* on behaviour and life history measures, *L. terrestris* hatchlings were reared in a *Monocystis* free environment and subsequently infected with *Monocystis* via controlled feeding of spores. Uninfected focals were paired with infected or uninfected (control) partners and mating behaviour was observed with infrared time-laps video recording. To control for substantial differences in life-history,

two cohorts of worms with approximately the same age were used for this no-choice experiment.

Pair or no-choice tests are indicated when group behaviour might disturb mating behaviour of the focal (Rundle and Schluter 1998; Shackleton, Jennions et al. 2005) Several special characteristics of hermaphrodites favour a no choice test over group experiments (Facon 2006): First, in groups with more than two animals, all potential partners can, unlike in gonochorists, mate with each other. Copulations between non-focal individuals may affect mate choice behaviour of the focal, and disentangling these effects is statistically challenging or even impossible. Also, potentially disturbing interactions of a third individual with a mating couple have been observed frequently (Chapter IV) in *Lumbricus terrestris*. Second, a large group is only required if animals sample and remember their potential choices, which is highly unlikely in earthworms (see Häderer, Werminghausen et al. 2009 for an example of lack of mate discrimination *in B. glabrata*) A third practical reason is the relatively homogenous outer appearance of most hermaphrodites. In contrast to separate sexes where males and females are usually easy to distinguish, the danger of losing data points due to missing IDs is much higher (but see Webster, Hoffman et al. 2003 for an elegant experimental set up in snails) especially when individuals cannot be marked (but see Janicke, Kesselring et al. 2012).

MATERIALS AND METHODS

Raising uninfected worms

Cocoons were obtained from purchased earthworms of Canadian origin (www.hw-terra.de, HW-Terra KG, Wirtsgrund 3 91086 Aurachtal) starting in December 2008. After removing all soil particles, cocoons were kept individually in six well plates on moist tissue paper. To prevent hatchling escape, the wells were sealed with parafilm. Moist tissue paper provides almost 100 % humidity and was also consumed by hatchlings. Incubation parameters were 15°C and complete darkness. Cocoons were checked every three days and hatchlings were transferred to individual 200 ml plastic containers half filled with substrate. The substrate, loamy garden soil, was prepared to be free from *Monocystis* spores by heat-drying (**Figure 1**), and subsequently rewetted and mixed with peat moss. Peat moss keeps moisture and prevents fungal growth. This substrate was exchanged once during the rearing phase. Hatchlings were fed with two spoons of dried, rewetted and shredded horse dung every four weeks.

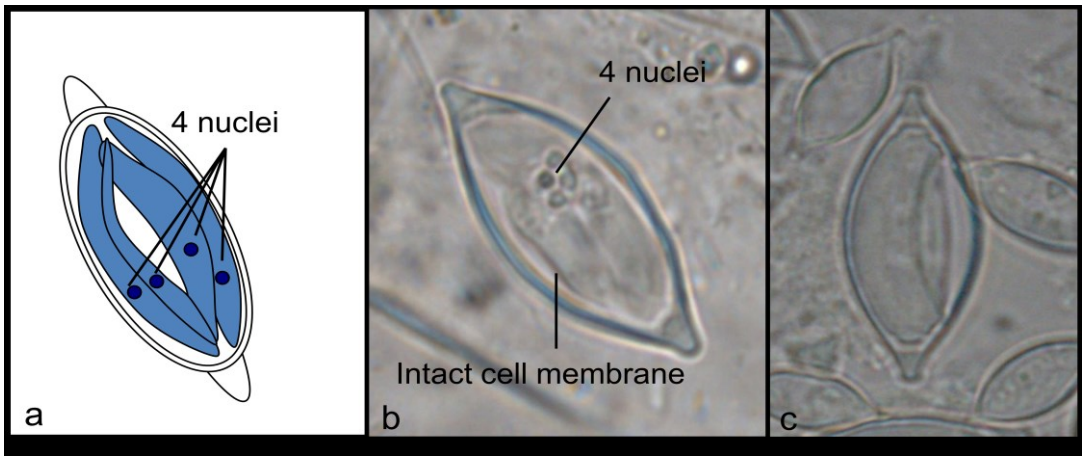

Figure 1 Killing of *Monocystis* spores via heat-drying
a Scheme of a *Monocystis* spore cell. Each spore contains 4 sporozoites.
b Light microscopy of live (infective) *Monocystis* spore (4–5 x 9–12 µm)
c Dead *Monocystis* spore after heat-drying. Nuclei and cell membranes are no longer visible.

Controlled infection

The first cohort of 51 earthworms reached sexual maturity, indicated by a visible clitellum, in December 2009, a second cohort with 132 individuals was used for the second run in March 2010. By using specimen with the same

age, I avoided confounding age-dependent mate choice, which was reported for other hermaphrodites (Hermann, Genereux et al. 2009). Earthworms were photographed under standard light conditions. After a short sedation on ice, pictures were taken from the anterior ventral region, the ventral clitellum and the ventral tail tip. Earthworms were then weighed and placed into individual housings as described in (Field and Michiels 2005). For hygienic reasons, the individuals´ foraging surfaces were not covered with packed soil. The food was directly placed onto the plastic surface, which was sprayed with deionised water every second day. Earthworms were kept in a climate chamber at a 11:13 day/night cycle at 15°C and 80 % humidity to acclimatize to their housings. During this period, they were fed twice with one teaspoon of prepared horse dung. After the acclimatization, the plastic bottles with the feeding surface were cleaned carefully to remove all remaining food items.

For the controlled infection, I decided against force feeding (Field and Michiels 2006) as internal damage cannot be ruled out. Instead, I presented the earthworms with a fixed volume of feeding solution consisting of fish food and *Monocystis* cysts out of a cocktail straw. Production of a standardised feeding solution was achieved by decontaminating fresh cysts followed by counting and appropriate dilution in fish food: The seminal vesicles of five freshly killed specimen were dissected out and pooled into a 1.5 ml Eppendorf cup. 200 µl of Ringer solution were added and the cyst solution was sonicated 4 seconds to homogenize. To remove bacteria and sperm cells, the solution was bleached by adding 500µl of bleach and 500µl of NaOH. Bleaching is a standard method to eliminate contaminations of earthworm tissue and potentially harmful bacteria (Stiernagel 2006) but does not harm the cysts (Milinski and Bakker 1990). After vortexing, the solution was centrifuged for 10 min at 14000 rpm. The supernatant was discarded and the pellet was redissolved in 1000 µl Ringer solution to wash out the remaining bleaching solution. After another centrifugation for 10 min at 14000 rpm, the supernatant was again discarded and the pellet was redissolved in 150 µl Ringer solution. A subsample of this cyst solution was used to prepare a 1:10 dilution with Ringer solution. This diluted cyst solution was then used to estimate the cyst concentration using a Thoma counting chamber (depth 0.1 mm).

A 15 ml Falcon tubes was filled with 1.2 g of fish food for herbivorous fish. The final amount of liquids added to the fish food was 2650 µl. Before adding the cyst solution, the content of the tube was vortexed for 30 s and then sonicate three times for 20 s to homogenize the fish food. As one such Falcon tube is enough to infect 25 earthworms, I added the volume of cyst solution for 2 500 000 cysts and adjusted the amount of Ringer solution accordingly. For the sham treatment, the same feeding solution but without cysts was prepared.

The bottom of the falcon tube was cut with a scalpel (**Figure 2a**) and the tip of 5 ml syringe was inserted so that the feeding solution could be filled into the

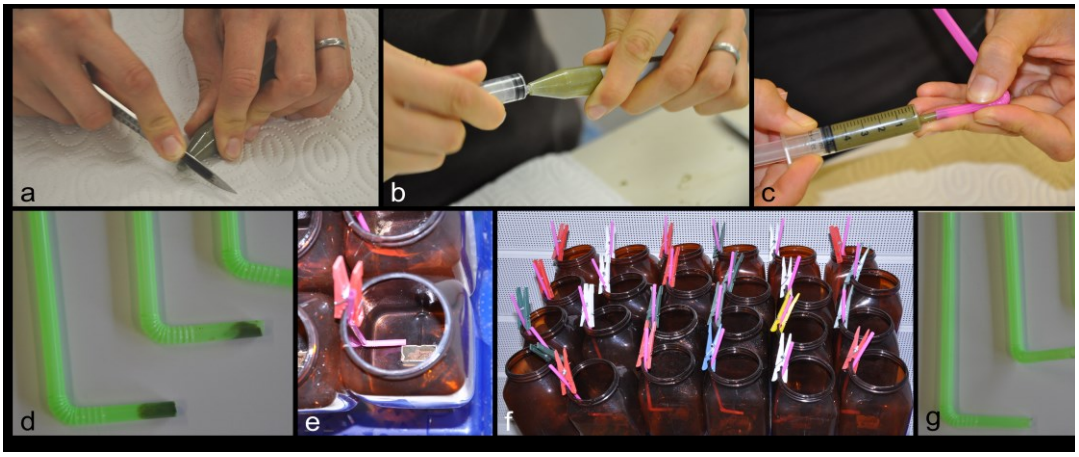

Figure 2 Preparation of cocktail straws for controlled infection.
a Cutting off the tip of a 5ml Falcon tube filled with feeding solution.
b Insertion of syringe into the tip.
c Filling of cocktail straws.
d Prepared cocktail straw.
e Single worm housing with cocktail straw above burrow entrance.
f Box with Randomised Block Design of infected/uninfected worms.
g Straw that was emptied by earthworms over night.

syringe (**Figure 2b**). After removing air bubbles the syringe was used to fill 100 µl of the feeding solution containing 100 000 cysts, into the tip of a cocktail straw (**Figure 2c, d**). In the evening, these straws were then fixed to the edge of the plastic bottles with a clothes-peg such that the end with the feeding solution was right above the burrow entrance (**Figure 2e, f**). Straws were removed every morning and checked for the presence or absence of the feeding solution (**Figure 2g**). In pre-experiments, I videotaped feeding behaviour and observed that earthworms either ingest the complete feeding

solution or ignore the food. Earthworms that fed did not receive another straw. The controlled infection phase lasted for 14 consecutive days (**Figure 3**). Earthworms that did not feed out of the straws during this phase were excluded from the study.

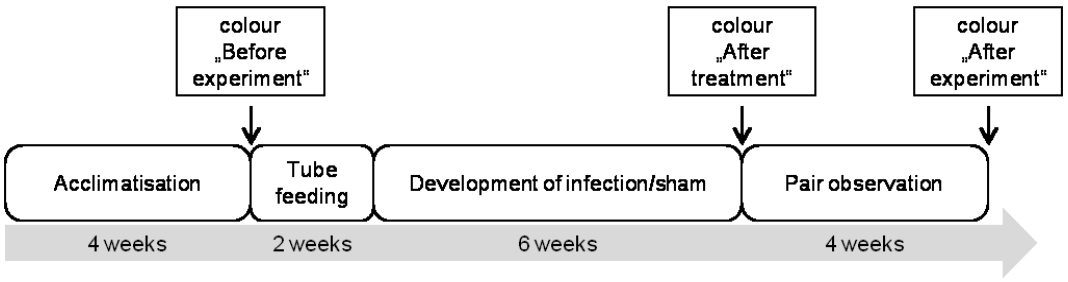

Figure 3 Timeline of experimental set-up. Arrows indicate time points where worms were weighed and fotographed.

Earthworms were kept another six weeks in their individual housings to allow the development of an infection (**Figure 3**). After that, three earthworms of each treatment were sacrificed and stored in 70 % ethanol over night. The seminal vesicles were dissected out, weighed and filled with the double amount of Ringer solution. After homogenization with a sonicator, a 1:10 dilution was used to determine the cyst concentration. As expected, earthworms that received the infection treatment harboured on average 12.000 cysts/µl, whereas the control treatments were *Monocystis* free.

Observation of pairs

Earthworms were then removed from their burrows, washed, weighed, photographed as described above and then placed back into their burrows. To avoid interference of size-assortative mating (shown in Michiels, Hohner et al. 2001) with the expected parasitic effects, pairs were assigned with the following rules:

(1) The weight difference between the partners should be minimized.

(2) The treatments should not differ in mean pair weight.

(3) In the uninfected (U) X infected (I) pairs, equal numbers of pairs with the infected or the uninfected partner being bigger should be achieved.

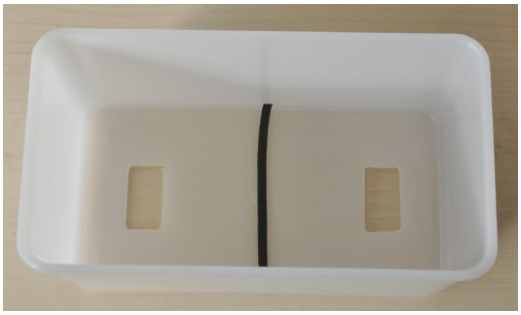

Figure 4 Plastic box with surface for pair observation and two holes to insert cable duct burrows.

Pairs were then assigned to two boxes in a Randomised Block Design, twelve pairs in each box. The single housing was replaced by a plastic box with two rectangular holes in the bottom (**Figure 4**). The holes fit exactly the distance between the burrow entrances of a pair. The centre between two burrows was marked with a 3mm strip of black, non-reflecting duct tape. The lids of the plastic boxes (not shown in **Figure 4**) were cut out to leave a 2 cm wide frame. This overhang is necessary to prevent escapes. A box containing 12 pairs was then observed with a separate infrared video camera. Time lapse recording intervals were 1 frame / 10 s. Earthworm surface behaviour was recorded for 28 nights in Run 1 and 24 nights in Run 2. During this period, I checked the videos every morning to make sure that every individual stayed in its burrow. Earthworms that were stranded on the surface were placed back into their burrows. The surface was sprayed with water daily, and earthworms were fed with one teaspoon of shredded horse dung in the two opposing corners of their boxes once a week.

Quantification of infection

After the pairing phase, earthworms were removed from their burrows, washed, weighed and photographed again. They were then sacrificed in 70% ethanol, cut in two parts behind the clitellum and stored in 70% ethanol in 50ml Falcon tubes for 24h at room temperature, then at -20°C for long term storage. After defrosting, each front part was opened dorsally with a scalpel. The dorsal body wall was pulled to either side and fixed with pins on a wax plate. The two pairs of spermathecae (Receptaculae seminis) on the ventral side were removed and placed together into a 1.5 ml Eppendorf tube with 400 µl of 100 % ethanol. The three pairs of seminal vesicles, located above the spermathecae and in the region directly behind from segment nine to 13, were removed with a pair of eye scissors and placed in a 1.5 ml Eppendorf

cup. After weighing them on a precision scale (± 0.1 mg), 2 µl of invertebrate Ringer solution (see appendix) were added for each mg of tissue for storage and *Monocystis* count. Spermathecae and seminal vesicles and tissue samples were then stored at -20°C.

Seminal vesicles were defrosted before use. They were then homogenized with a hand homogenizer (Roth) in a drilling machine until no more clumps were visible. Further homogenization was achieved via sonication with an ultrasound Sonicator (UW 2070, Bandelin electronic, setting: 1 second, cycle 4, 33 %). The machine was cleaned with ethanol after every sample. After vortexing for 10 s, a 1:10 dilution with Ringer solution was prepared. This dilution was again sonicated and vortexed as described above and then used to prepare another 1:10 dilution, leading to a final 1:100 dilution. The concentration of *Monocystis* sporocysts was determined with a big Thoma chamber (depth 0.1 mm, Thoma CE, Superior Marienfeld, Germany) under a phase contrast microscope (DM 5000 B, Leica) with magnitude 40 x 10. *Monocystis* sporocysts can easily be recognized by their fusiform shape. For each sample, two separate 1:10 dilutions were counted. The counting protocol was modified after (Field, Schirp et al. 2003). To estimate the sperm concentration, I counted sperm cells of three separate 1:100 dilutions in a small Thoma chamber (depth 0.02 mm, Thoma CE, Superior Marienfeld, Germany) under a phase contrast microscope (DM 5000 B, Leica) with magnitude 40 x 10.

Reproductive Output

After the experiment, all cable ducts were randomised. The substrate inside the cable ducts was individually washed out and gently passed through a mesh (width 4 mm) under the water jet of a Gardena® hand sprinkler. Cocoons were sorted out and counted.

Video analysis

Videos were analysed using the program Iguard® Player. To determine the mating latency, I also recorded the time of the first physical contact between partners and subsequently noted date, time and duration of the following

copulations. A copulation started when the partners had assumed the typical S-shaped position and ended when the two worms were completely separated. Burrow visits were only recorded when worms stuck their anterior part into the other partners burrow until the clitellum was barely visible. I recorded number, length and direction of burrow visits from the focal´s point of view. Additionally, extraordinary behaviour such as mating outside of the burrow and unilateral matings were noted.

Colour analysis

Pictures were randomised prior to analysis, and spectral parameters were measured using the method described in (Field, Schirp et al. 2003). Briefly, using the Adobe® Photoshop®, a 10 x 10 pixel square of a non-reflecting body part was selected, homogenized with Gaussian blur and colour characteristics were determined with the colour picker tool which measures hue, brightness and saturation. This was repeated three times for each picture. In cases where the hue value transitioned the 0/360° mark, it was converted into a corresponding negative value. Results were then averaged.

Several spectral parameters were strongly correlated, so I fused them using a Principal Component Analysis and used the first two principal components (PC) for further analysis (**Table 1**).

PC [time point]	Run 1				Run 2			
	%	X^2	df	p	%	X^2	df	p
1 [Before experiment]	23.0	89.11	44	< 0.001	32.1	230.66	44	< 0.001
2 [Before experiment]	19.2	71.77	35	0.0002	17.7	132.31	35	< 0.001
1 [After treatment]	29.6	88.47	44	< 0.001	31.1	209.27	44	< 0.001
2 [After treatment]	17.2	51.43	35	0.0362	15.1	118.67	35	< 0.001
1 [After experiment]	28.7	121.78	44	< 0.001	29.5	194.5	44	< 0.001
2 [After experiment]	20.2	87.47	35	< 0.001	17.9	47.43	35	< 0.001

Table 1 Results of Principle Component Analysis of spectral parameters.

To interpret the colour parameter pattern, a parameter was said to load on a component if the factor of the Eigenvector was 0.40 or greater for that component, and was less than 0.40 for the others (Andersson and Simmons 2006). Resulting main components are listed in **Table 2**. In order to compare PCs of different time points, I used the formula defining PCs after treatment to calculate PCs before and after experiment.

PC [Time point]	Run 1	Run 2
1 [Before experiment]	Hue front Saturation front Saturation tail (-)	Saturation front Brightness front Brightness clitellum
2 [Before experiment]	Saturation front Hue clitellum Brightness clitellum	Hue front Hue tail Saturation tail
1 [After treatment]	Saturation front Brightness front Brightness clitellum	Brightness front Brightness clitellum
2 [After treatment]	Hue tail Saturation tail	Hue tail Saturation tail
1 [After experiment]	Saturation front Brightness front Saturation clitellum	Saturation front Brightness front
2 [After experiment]	Hue tail Saturation tail	Hue clitellum Brightness clitellum

Table 2 Main components of the Principal Component Analysis.

Data analysis

Data were analysed using JMP ® 9.0.1 (SAS Institute Inc.). Data of the first run were used as preliminary data due to small sample size and a difference in experiment duration of 4 nights. A summary of the results of Run 1 can be found in **Table 6.** As individual data of pairs are not independent, I used only data of infected and uninfected non-focals to analyse the fitness effects of *Monocystis* (**Figure 5**).

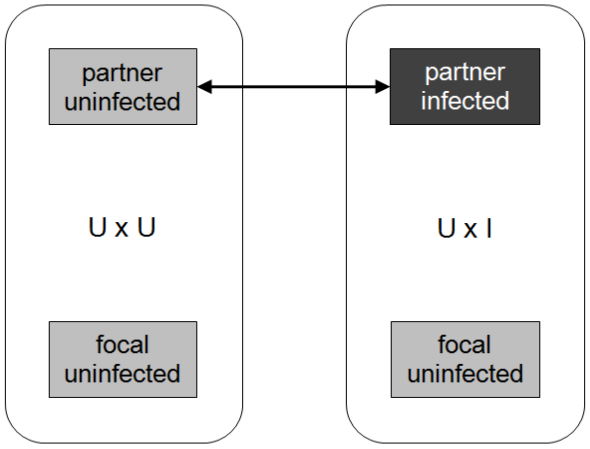

Figure 5 Experimental set up for pair observation. Arrow connects individuals that were compared for parasite effects.

Successful Infection

To my knowledge, this is the first study that successfully used controlled feeding to infect *L. terrestris* with *Monocystis* (**Figure 6**). The new method I developed here has two major advantages: First, the cyst solution is cleaned from other potentially harmful pathogens, e.g. bacteria and nematodes via bleaching. And second, controlled feeding does not cause internal damage, which is likely to occur when worms are force-fed with a silicon tube (Field and Michiels 2006) Internal damage and bleeding should be avoided when behaviour is recorded after infection.

The new infection method proved successful. Earthworms in the infection group showed considerably high, but quite variable concentrations of *Monocystis* spores, ranging from 1.1×10^3 to 34.5×10^3 spores/µl ($\bar{x} = 13.1 \times 10^3 \pm 9.7 \times 10^3$ spores/µl). Most control animals stayed uninfected (**Figure 6**) but three infected earthworms of the control group had to be excluded for further analysis. *Monocystis* spore concentrations did not differ between runs (*Wilcoxon test, Z = 0.51,* $n_{run1} = 9$, $n_{run2} = 34$, $p = 0.50$).

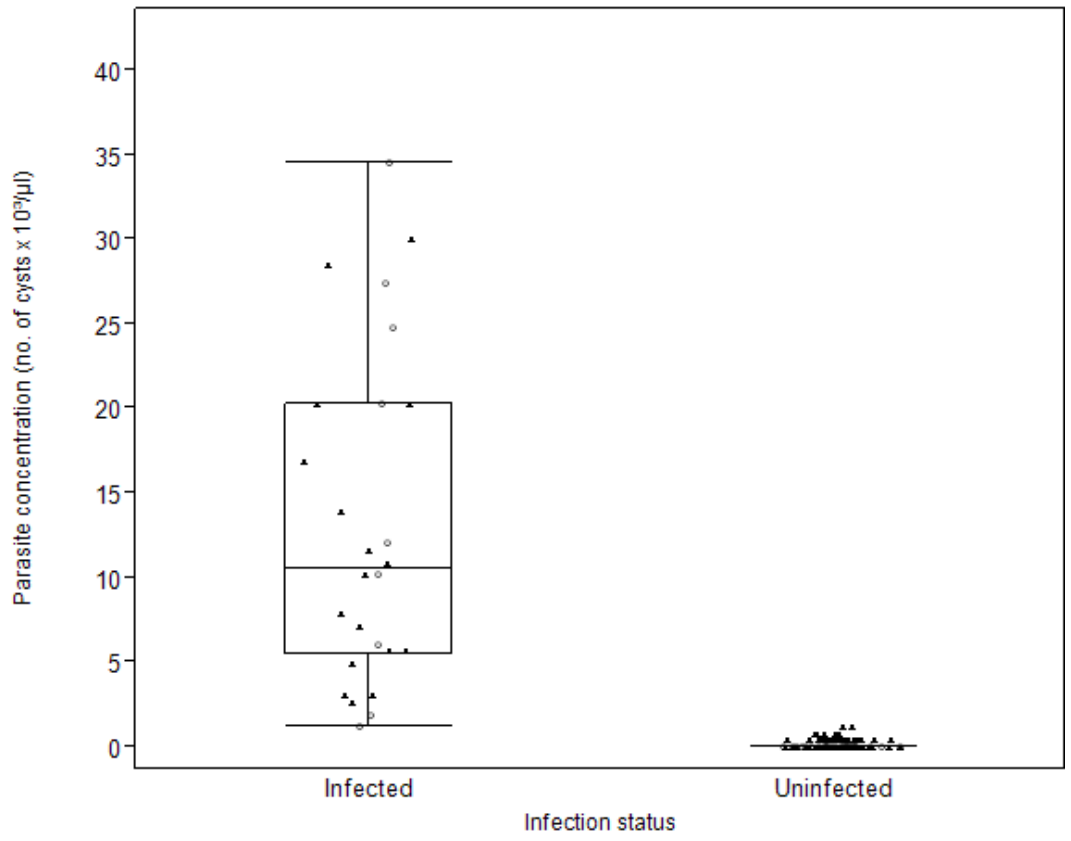

Figure 6 Boxplots for parasite concentrations in infected and control earthworms. Circles represent run 1, triangles represent run 2. Groups are significantly different (*Wilcoxon test, Z = 9.25,* $n_{infected} = 26$, $n_{uninfected} = 114$, $p < 0.001$).

Extraordinary mating behaviour

Throughout the pair phase of the experiment Run 2, I could observe copulations where one partner crawled out of its burrow during or even before the mating. In total, I observed 7 matings with one partner completely outside of its burrow. Out of those 7 earthworms, 4 were infected with *Monocystis*, 1 was a partner of an infected individual and 2 were uninfected. Those matings did not last much shorter ($\bar{x}_{\text{mating outside}}$= 2 h 34 min) than the overall average

mating duration of 2 h 41 min. I could observe that most worms found their way back to their burrows and continued feeding normally, so I included those matings in the analysis. One should, however, reconsider the statement of (Michiels, Hohner et al. 2001) that earthworms get pulled out of their burrows and are stranded on the surface. This may hold only for long-distance copulations.

Two pairs performed what seems to be a unilateral mating with only one worm attached with its male pores. These two matings were only slightly shorter (2 h 17 min and 2 h 03 min) than average and were therefore included in the analysis.

RESULTS

Parasite costs on male and female reproductive functions

Here, I evaluate only results for run 2. A summarized comparison with results of run 1 can be found in **Table 6**.

Should *Monocystis* nourish on host tissue for reproduction, I would expect to find direct effects on the organ it propagates in. However, seminal vesicles were not smaller in infected individuals (*t-test, t* = -0.75*,* $n_{infected}$ *= 17,* $n_{uninfected}$ *= 30, p = 0.46)*. Infected earthworms could suffer from parasitism in male reproductive tissue such that sperm production is reduced: I could find a trend that infected individuals have a lower sperm concentration (**Figure 7**).

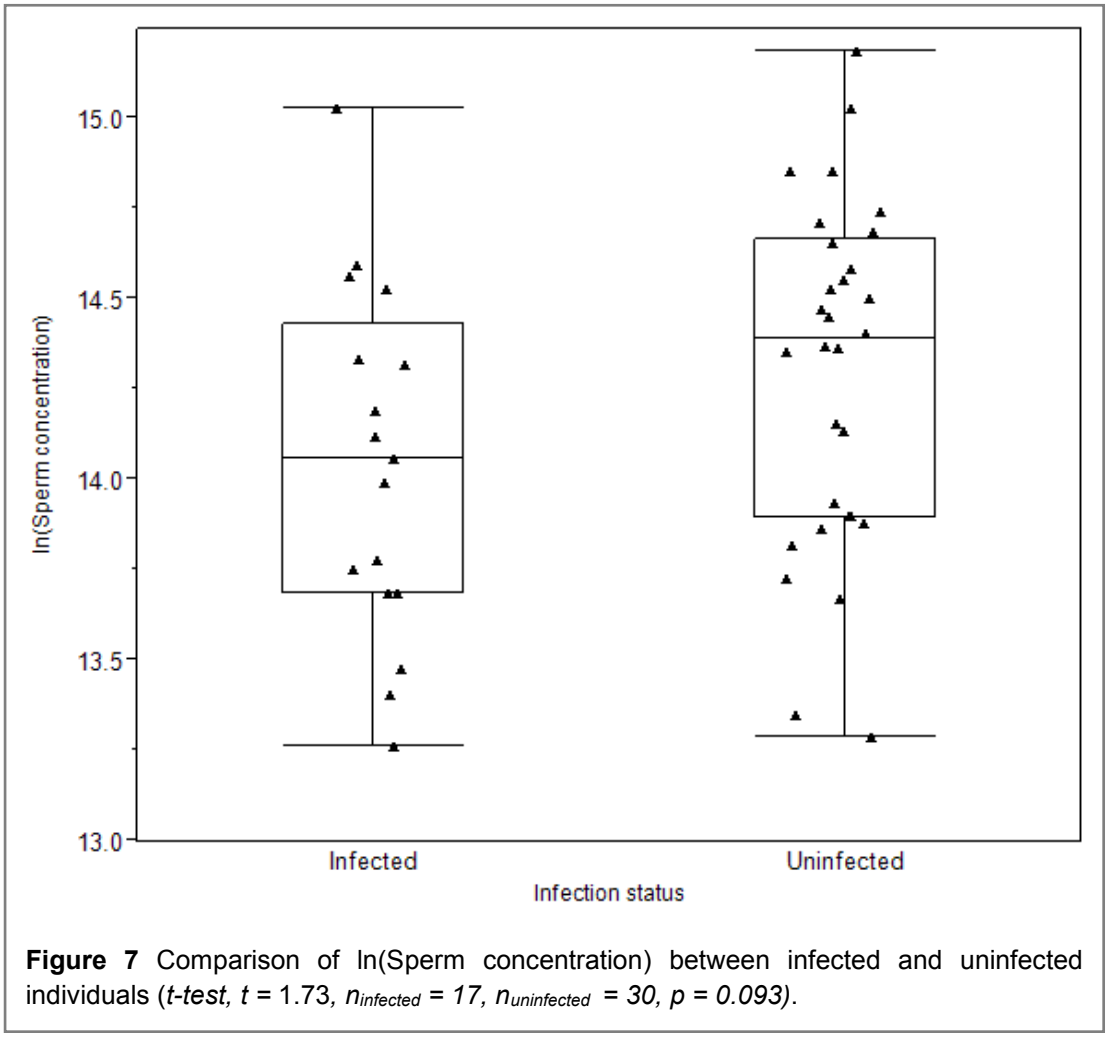

Figure 7 Comparison of ln(Sperm concentration) between infected and uninfected individuals (*t-test, t* = 1.73, $n_{infected}$ *= 17,* $n_{uninfected}$ *= 30, p = 0.093)*.

Fitness costs on the female function are often more systemic and affect female reproductive output via reduction in body weight. In my study, I could not detect a slower growth of infected individuals (*t-test, t* = -0.97, $n_{infected}$ *= 17,*

$n_{uninfected}$ = *30,* p = 0.338*)*. Also, *Monocystis*-infected earthworms did not produce fewer cocoons than uninfected conspecifics (*t-test,* t = -1.32*,* $n_{infected}$ = *17,* $n_{uninfected}$ = *30,* p = 0.194).

Parasites influence mating behaviour

I detected several effects of *Monocystis* infection on *L. terrestris* mating behaviour: When looking at burrow visits as a premating behaviour, most earthworms of my study performed no or one burrow visit. When comparing focal burrow visiting activities, I found that the proportion of performing focals was greater in U x I pairs (**Figure 8**). That means that focals paired with an infected individual are more likely to perform one or more burrow visits than focals paired with uninfected individuals.

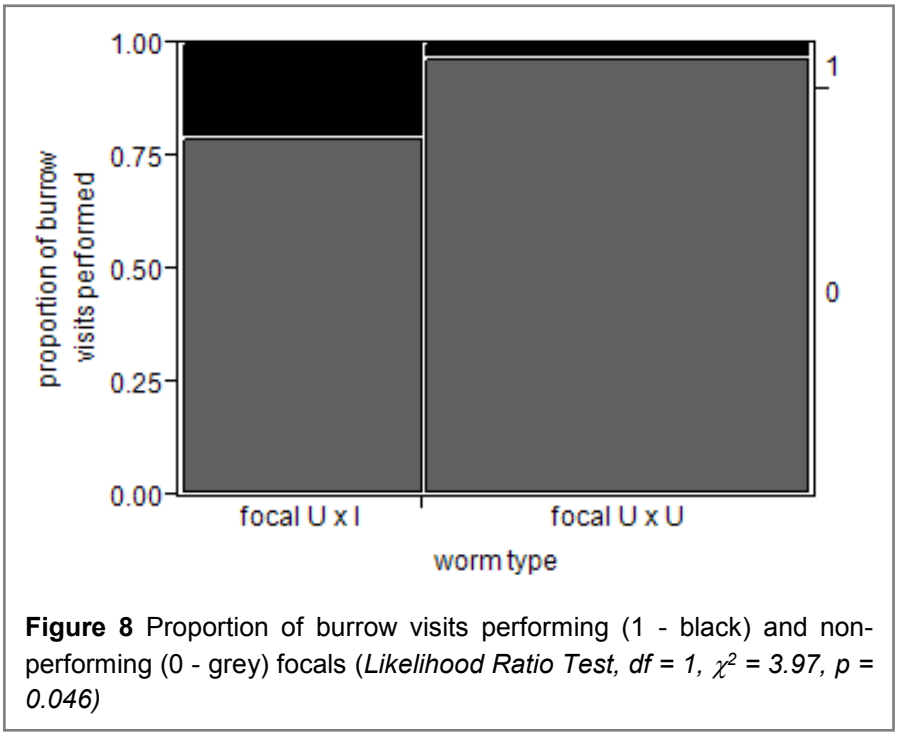

Figure 8 Proportion of burrow visits performing (1 - black) and non-performing (0 - grey) focals (*Likelihood Ratio Test, df = 1,* χ^2 *= 3.97, p = 0.046)*

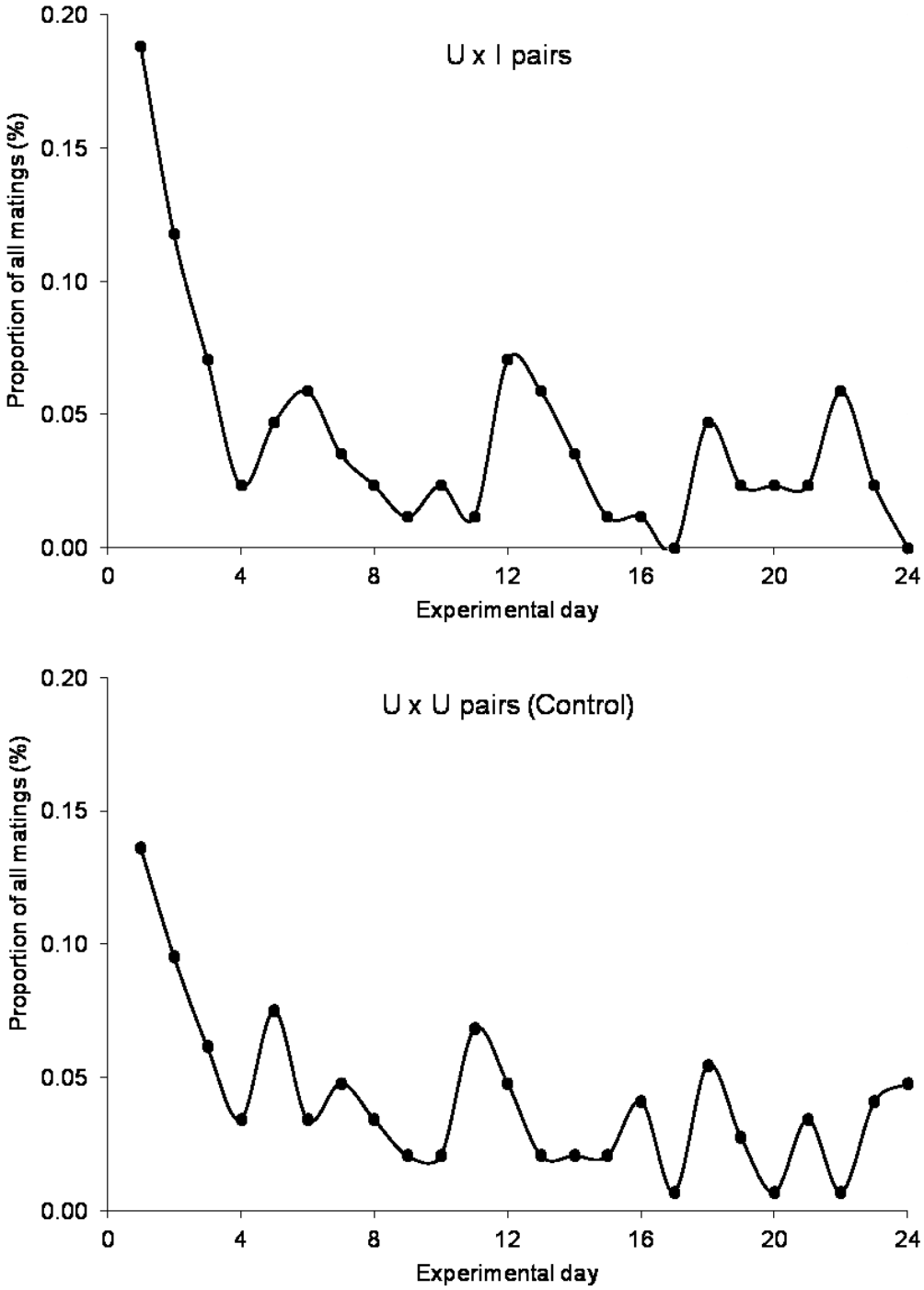

Figure 9 Timeline for daily mating rates in both treatment groups.

In both treatment and control groups, mating rates were highest in the first week of the pair phase and stayed constant afterwards (**Figure 9**). I therefore examined start, duration and interval between the first two copulations of every pair in more detail. For parasite-mediated mating behaviour, I expected that U x U pairs would start copulating earlier, have a higher mating rate and

take shorter time to mate again. U x I pairs did not differ from U x U pairs in those mating parameters (**Table 3**). Mating latency, however, increased with increasing parasite concentrations of the infected partner in the U x I group (**Figure 10**).

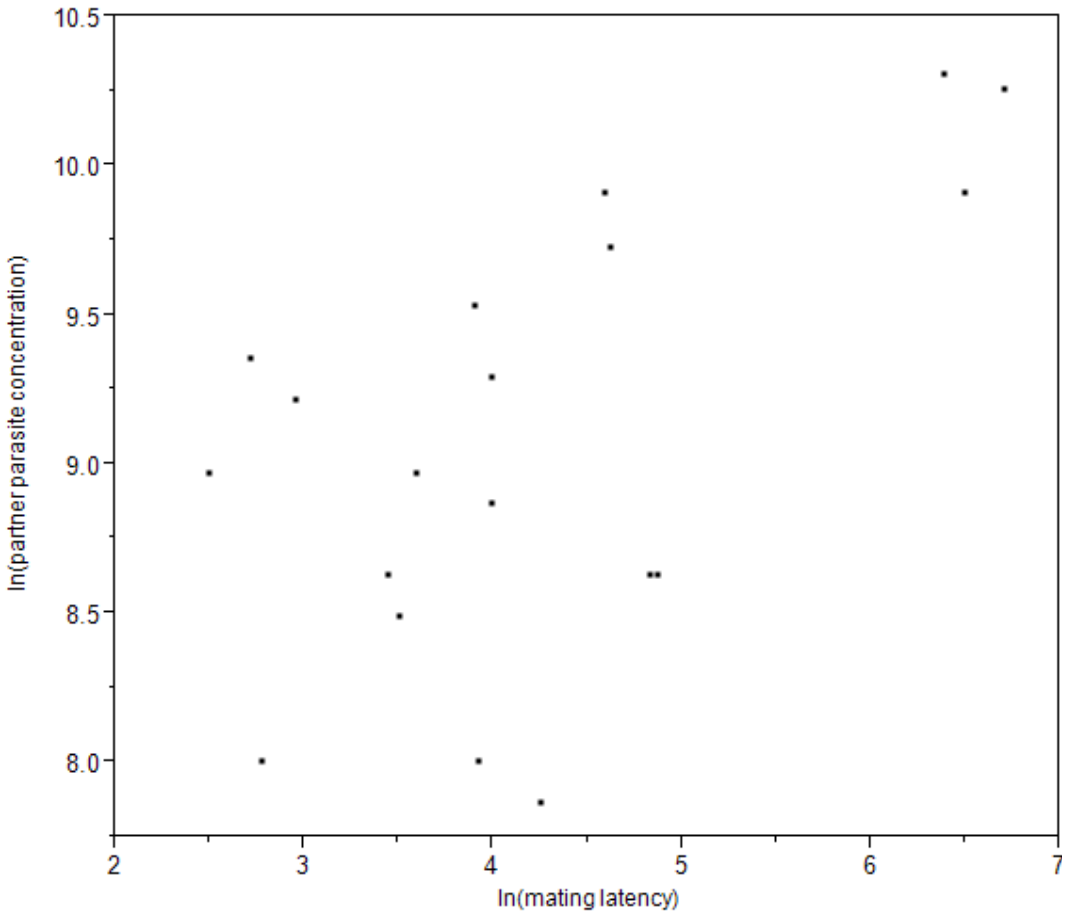

Figure 10 Correlation between ln(mating latency) and ln(partner parasite concentration) (*Pearson correlation, r = 0.58, n = 17, p = 0.0094*).

Parameter	Experimental Group				Test statistics	
	U x I n = 17		U x U n = 30			
	$\bar{x}$	SD	$\bar{x}$	SD	t	p
Mating rate [matings/week]	0.79	0.26	0.85	0.25	0.88	0.39
Mean mating duration [min]	160	12	161	9	0.54	0.60
Mating latency[#] = time between 1st contact and 1st mating without light phases [min]	67	101	74	140	0.26	0.80
Remating time[#] = time between start of 1st and start of 2nd mating without light phases						
[min]	1339	1789	1636	1241	0.46	0.65
[nights]	2.02	2.7	2.48	1.88		

Table 3 Results of comparison of mating parameters in U x I and U x U (control) pairs (*t-test*)
[#] data were ln(x) transformed.

Spectral measurements, Monocystis infection and time effects

I suspected that infection with *Monocystis* changes the outer appearance of *L. terrestris.* Unfortunately, individuals of the infection and the control group differed in colour prior to the experiment (PC1, *t-test, t* = -2.21*,* $n_{infected}$ *= 17,*

$n_{uninfected}$ = *30, p* = 0.03), so I first focused on within-worm effects (time effects) (**Table 4**).

	Infected individuals (n = 17)			Uninfected individuals (control, n = 30)		
	Before experiment	After treatment	After experiment	Before experiment	After treatment	After experiment
PC1: front brightness & clitellum brightness						
$\bar{x}$	-0.97	0.90	-4.26	0.11	0.10	-3.78
t	*0.27*	*-14.03*		*0.27*	*-15.59*	
p	*0.79*	*<0.008***		*0.97*	*0.008***	
PC2: tail hue & tail saturation						
$\bar{x}$	-0.91	-0.05	0.62	-0.46	-0.08	0.67
t	*3.03*	*1.58*		*-1.48*	*3.25*	
p	*0.0395**	*0.13*		*0.15*	*0.0186**	

Table 4 Time effects for colour measurements of infected and uninfected individuals. (*Pairwise t-tests*, p-values are Holm-Bonferroni corrected.)

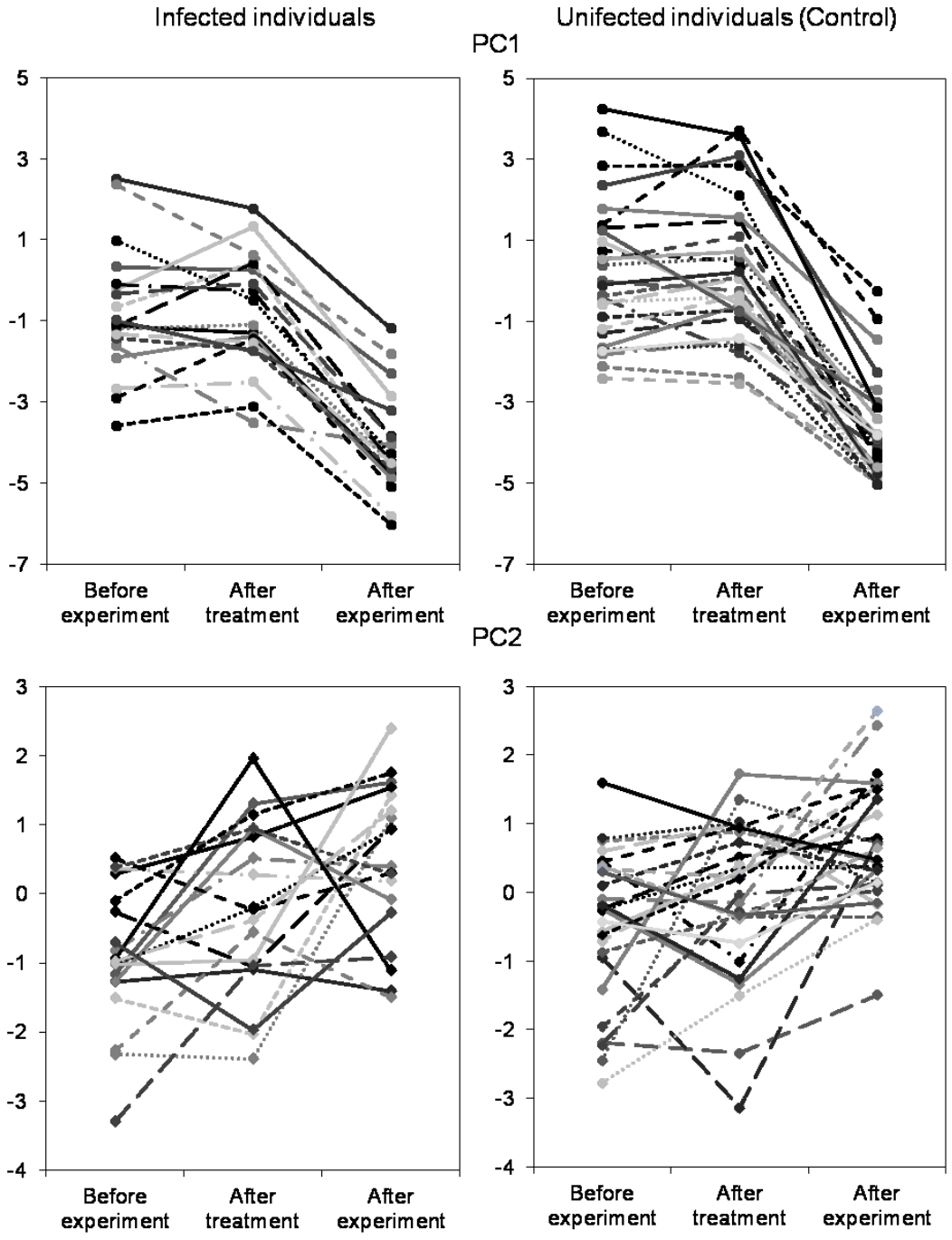

Figure 11 Pairwise comparisons within individuals of PC 1 and PC2 between different time points during the course of the experiment for both treatment groups. Different lines indicate different individuals.

I detected a shift in colouration in both infected and control animals over the course of the experiment (**Figure 11**). For the colour measurements after the experiment, PC1 massively dropped in both groups. I could only detect an increase in PC2 after infection with *Monocystis* in the treatment group while the colour of control animals did not change in a particular direction.

Could earthworms use colours as indirect mate choice criteria?

If earthworms use colour as indirect parasite-mediated mate choice criteria, there should be a connection between colour and parasite burden. I could not find a direct correlation between ln parasite concentration and PC1 or PC2 (*Pearson correlation r = -0.0577, p = 0.82 and r = 0.37, p= 0.13, respectively*), but when looking at mating behaviour, mating latency in U x I pairs increased with increasing PC2 of the infected partner (**Figure 12**). To determine which measurement would give a better explanation for mating latency, I compared two ANOVAs: One containing only parasite concentration, and one with colour and parasite concentration (**Table 5**). The parasite model has a highly significant fit. Including PC2 does not improve this model: Although the p-value for ln(parasite concentration) is slightly lower, the F value of the combined model is getting weaker. I therefore infer that colour does not play a major role to explain mating latency.

Model	Overall Model fit	Variables	Model estimates	t	p
Parasites	F = 8.77 p = 0.0097*	Intercept	-5.10	-1.62	0.13
		ln(parasite concentration)	1.02	2.96	0.0097*
Parasites & colour	F = 6.78 p = 0.0096*	Intercept	-4.19	-1.21	0.25
		ln(parasite concentration)	0.92	2.47	0.00284*
		PC2	0.39	1.72	0.11

Table 5 ANOVAs to explain mating latency with (1) parasites or (2) parasites and colour.

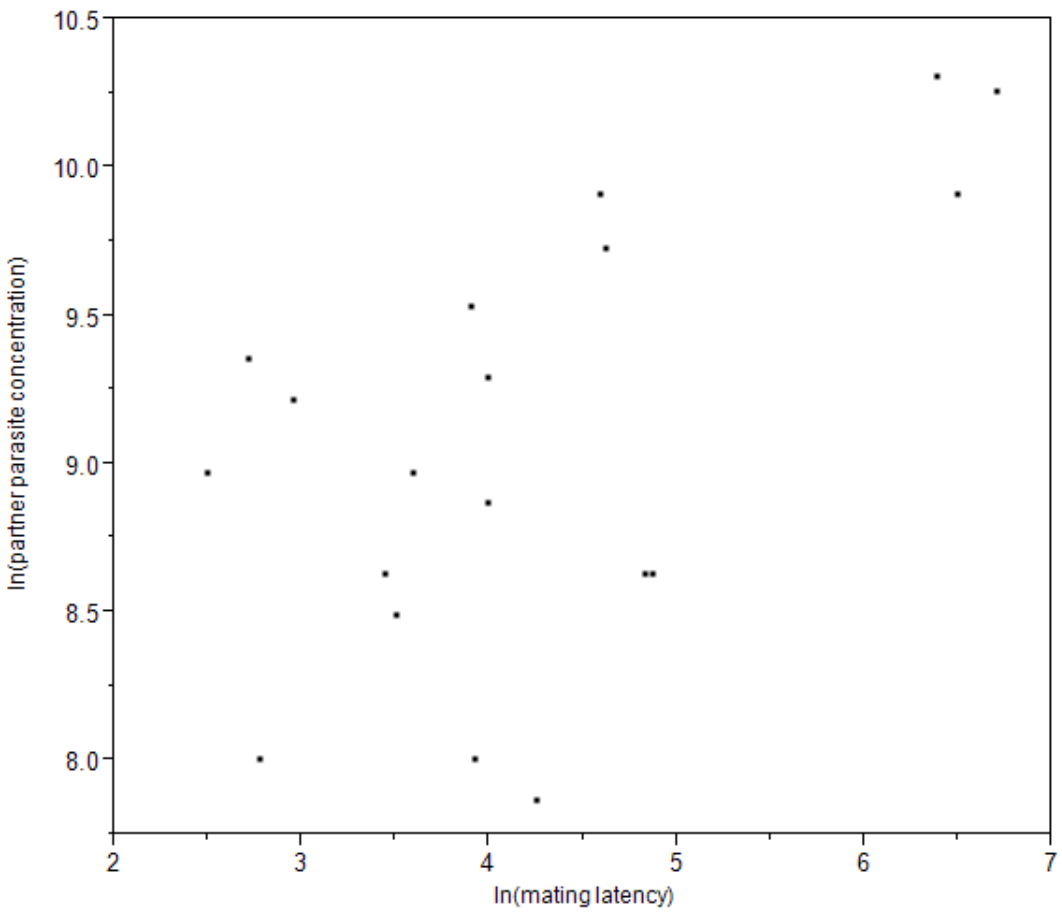

Figure 12 Correlation between PC2 of infected partner and ln(mating latency) of U x I pairs (*Pearson correlation, r = 0.50, p = 0.0329).*

	Run 2			$n_{infected}$=17		$n_{uninfected}$=30		Run 1			$n_{infected}$=9		$n_{uninfected}$=13	
Parameter	t	df	p	$\bar{x}$	SD	$\bar{x}$	SD	t	df	p	$\bar{x}$	SD	$\bar{x}$	SD
Growth after infection (g/day)	-0.97	25	0.338	-0.02	0.03	-0.03	0.02	0.79	19	0.437	-0.005	0.015	0.00	0.017
Cocoon production#	-1.32	39	0.194	5.21	2.71	4.18	2.71	1.13	13	0.278	4.66	2.27	5.70	1.85
Vesicle weight (mg)	-0.75	29	0.46	48.76	13.89	45.77	11.73	-1.93	12	0.077	63.22	13.52	53.38	8.58
Sperm concentration# (cells/µl)	1.73	33	0.093	1.30 x 10^6	0.62 x 10^6	1.62 x 10^6	0.81 x 10^6	1.85	17	0.082	1.45 x 10^6	0.32 x 10^6	1.96 x 10^6	1.11 x 10^6

Table 6 Comparison of individual data for infected and uninfected *L. terrestris* for run 1 and run 2 (*Student-T tests*).

#data were ln(x) transformed for analysis. For better illustration, $\bar{x}$ and SD were retransformed in this table.

DISCUSSION

This is the first study that examines parasite-mediated mate choice in a reciprocally mating hermaphrodite. My results indicate that parasite-mediated mate assessment prior to copulation indeed plays a role in *L. terrestris.* The use of hand-reared, uninfected individuals and controlled infection allows for detailed evaluation of parasite-induced changes in mating behaviour.

Parasite costs on male and female reproduction

Monocystis infections seem to influence the male function of *L. terrestris*: I could show a trend that *Monocystis* has a direct negative effect on sperm cell abundance in the seminal vesicles (**Figure 7**). This could merely be an effect of spatial displacement, but is also likely to result from trophic interactions: Schmidt and Roberts (2005) describe that during the *Monocystis* life cycle, sporozoites penetrate the seminal vesicles and enter sperm-forming cells of the vesicle wall. In the following growth phase, they destroy developing spermatocytes. Tissue destruction is also known in other apicomplexan species that are closely related to *Monocystis*: For example, in the well-studied *Plasmodium-Anopheles* system, host midgut epithelia cells are destroyed by invading oocytes (Zieler and Dvorak 2000) and *Cryptosporidium* sp. infests gut epithelia cells (O'Donoghue 1995) of mammals. When additionally considering that immune cells (coelomocytes) of *L. terrestris* are able to recognise *Monocystis* as non-self (Reinhart and Dollahon 2003), my results point strongly toward true parasitism. They are in accordance with the group study in chapter IV, where parasite and sperm cell concentrations are negatively correlated.

In hermaphrodites, female reproductive output is often coupled with body size. In my study, I did not find parasite effects in this direction, neither on growth nor on cocoon production. At first, this seems contradictory to other studies were *Monocystis* infection impeded growth, and size was correlated with cocoon production (Field and Michiels 2005). This study however did not use uninfected earthworms as control, but performed correlative analysis of data

from purchased earthworms with unknown origin. In my study, interpretation of results is clearer because all individuals were nearly the same age and shared a common rearing history.

It has been suggested that intermediate levels of virulence serve the castrating parasites most because it needs to balance transmission success and the possibility of host extinction (Jensen, Little et al. 2006; Szilágyi, Scheuring et al. 2009). In the light of my results, i.e. a negative effect on sperm cells, this could explain the rather weak fitness effect and high parasite prevalence in wild populations (Field, Lange et al. 2007). *Monocystis* and *L. terrestris* might be so closely adapted that they co-reproduce, as subadults harbor significantly less parasites than adults (Field, Schirp et al. 2003). These findings are also consistent with evidence for well-adapted, relatively harmless parasites in other Eugregarinorida and their insect hosts (Klingenberg, Leigh et al. 1997; Hecker, Forbes et al. 2002; Canales-Lazcano, Contreras-Garduno et al. 2005; Rodriguez, Omoto et al. 2007).

Parasite-mediated mate choice in L. terrestris

In my experiment, focals paired to infected partners more often engaged in burrow visiting behaviour (**Figure 8**). The function of burrow visits has so far been not completely clear: Worms could either visit partners to persuade their partners into mating or, on the contrary, carefully evaluate their mates. In other studies, visit frequency has been suggested as a measure for mating reluctance (Michiels, Hohner et al. 2001) in earthworms. Considering that *Monocystis* has negative effects on the male function, either by reduced sperm concentrations or by physical blocking of the vasa deferentia, this could have consequences for mating interactions: In a system where mutual insemination is the rule, but some individual are hampered in sperm donation, one should make sure that copulation is fairly reciprocal. Baur, Locher et al. (1998) reported for the land snail *A. arbustorum* that not all precopulatory interactions lead to pair formation, but when they do, sperm transfer is almost always reciprocal. A similar pattern can be observed in *L. terrestris*: Copulations do not necessarily follow after burrow visits. During precopulatory assessment, worms could touch reproductive structures such as the clitellum

and especially the glanular margins of the male pores to check for proper functionality (Cosín, Novo et al. 2011). One could imagine that blockage of the vasa differentia with *Monocystis* spores and successive swelling after immune reactions would be tangible through the soft epidermis. Precopulatory body scanning is only one explanation for the function of burrow visits. Other authors suggest that earthworms evaluate partner body size in order to avoid a tug-of-war in long distance copulations (Michiels, Hohner et al. 2001) or that they test the partner burrow´s microclimate as a suitable habitat for cocoon and hatchling development (Grigoropoulou, Butt et al. 2008; Cosín, Novo et al. 2011) The latter explanations are plausible, but do not give reason for repeated burrow visits. Repetitive behaviour is more likely when the partner is assessed in greater detail or as part of courtship.

In my case, uninfected individuals can be unwilling to mate with infected partners not only per se, but also on a quantitative scale: Mating latency, the time between first body contact and first copulation was negatively correlated with parasite concentration (**Figure 10**). This hints at a concentration effect of the parasite. Indeed, (Schmidt and Roberts 2005) propose that castration through blockage is more likely with high spore densities. Here, again, earthworms need to utilize mate assessment to gain information about parasite loads of their partners. Infected individuals were the only accessible partner so uninfected focals eventually agreed in mating with them, possibly because they were virgins and in the need of sperm.

Optimal mating rates and remote copulations in L. terrestris

Why didn´t I find a difference in mating duration or mating rate between U x U and U x I pairs (**Table 3**). For sure, sperm depletion cannot be the reason for these short remating times because *L. terrestris* is known to store and use sperm from a single mating for up to 6 month (Butt 1993). A possible explanation could be found in the unique form of sexual conflict in lumbricid earthworms: Partners inject each other with allohormones, which influence sperm uptake and induce a refractory period during which remating is suppressed (Koene, Pförtner et al. 2005). When allohormones are injected in every mating, it could become redundant to have an internal mating rhythm,

so earthworms could rather depend on a slow decomposition of allohormones to trigger remating. An astonishing similar overall mating rate (0.98/week) in the group observation (Chapter IV, data not shown) adds to the picture of unflexible copulation timing. While the spike in mating rate at the beginning of the pair phase (**Figure 9**) might result from mating eagerness due to mate novelty or virginity (Michiels and Bakovski 2000; Anthes, Putz et al. 2006b; Janicke, Kesselring et al. 2012), fixed copulation rates seem to be an expression of optimisation for the female function (Sprenger, Faber et al. 2008; Sprenger, Lange et al. 2011).

Additionally to constant mating rates, mating duration is constrained to 2-3 hours and worms appear to run on “auto-pilot” once both partners engaged in mating. Assumption of the typical S-shaped position is realized by the same body movements in all pairs. During the subsequent mutual insemination, the relatively complex genital structures have to align symmetrically. Accomplishment of this task is crucial for successful sperm transfer and following cocoon production and may facilitate the stabilizing evolution of such stereotypic mating behaviour. In the closely related leeches, mating behaviour is not induced by the brain, but by simple neurons and cannot be stopped once commenced (Wagenaar, Hamilton et al. 2010). Similarly, Koene, Jansen et al. (2000) found that evolution of brain areas that control copulatory functions is highly conserved in two gastropod subclasses.

How can earthworms perceive parasitism in their mates?

Both infected and control animals changed in colour during the course of the experiment (**Figure 11** and **Table 4**).In the pair phase, PC1 massively decreased in both groups. PC1 is positively correlated with front brightness (Eigenvector 0.49) and clitellum brightness (Eigenvector 0.53). This means that the front region of the worms got paler during the pair phase. The clitellum of virgin *L. terrestris* appears to be rather light, whereas mated individuals show substantial tissue damage in this area due to piercing with copulatory setae (Koene, Sundermann et al. 2002 and S. Weller, personal observations). The following healing process as well as cocoon production

might be the cause for colour changes. Colour could therefore be an indicator for both mating status and potential to produce cocoons in the near future.

PC2 increased for infected individuals after infection while control animals remained stable in their colouration. PC2 is positively correlated with tail hue (Eigenvector 0.69) and tail saturation (Eigenvector 0.60), so earthworm tails changed towards orange and have higher saturated colours. This could be due to a special immune function of earthworms: Non-self objects including all kinds of pathogens are encapsulated via melanisation into brown bodies and then subsequently moved towards the tail tip, where they are discarded via autotomy (Valembois, Lassegues et al. 1992; Valembois, Seymour et al. 1994; Field, Kurtz et al. 2004). In infected individuals, parts of the seminal vesicles were found melanised and separated from the main organs. In addition I found parasite cysts in dissected brown bodies from the tail tip. Conclusively, brown bodies resulting from immune reactions could tinge the tail tip darker. Clearly, the outer appearance of *L. terrestris* is associated with general health and mating status and might therefore provide a visual clue for potential sexual selection. Indeed, mating latency in U x I pairs increased with increasing PC2 of the infected partner. I further investigated the possibility of colour being a reliable signal for parasite burden. My model (**Table 5**) suggests that colour (PC2) does not account for increase in mating latency and that parasite concentrations directly affect the delays to copulation. One plausible explanation is that PC2 indeed reflects parasite loads via spectral measurements of the tail tip, but this part of the body remains anchored in the burrow during visits and mating, making “visual” perception with primitive photoreceptors (Hess 1925) impossible.

So far, little is known about the proximate mechanism underlying hermaphrodite partner evaluation. For sure, chemical compounds e.g. in shell and mucus play an important role (Webster, Hoffman et al. 2003; Schjorring and Jager 2007) and such chemical cues can be necessary for finding and attracting mates in earthworms (Golding and Olive 1978). On the physiological side, the prostomium is a sensory lobe stacked with chemoreceptors and other sensory cells (Wallwork 1983). Precopulatory visits

will involve contact of the prostomium with potential partners allowing for tactile and chemical evaluation.

Which sex exhibits choice? – Sex allocation and parasitism

I could clearly demonstrate parasite-mediated precopulatory mate choice in *L. terrestris*, but which sexual function is the choosing agent? Hermaphrodites are generally expected to choose for female fecundity (i.e. body size) via their male function (Leonard 2006; Anthes 2010), whereas choosiness for parasite resistance is predicted for the female function. From the perspective of the female function of earthworms, precopulatory parasite-mediated mate choice is especially advantageous. Unlike in other hermaphrodites, there is little scope for postcopulatory (i.e. female controlled) selection: Neither is sperm stored in different compartments (Sahm, Velavan et al. 2009; Novo, Almodóvar et al. 2010) nor is sperm digested in substantial amount (Jamieson, Fleming et al. 1982). Excessive precopulatory burrow visits to infected partners could therefore mirror preferences of the female function. On the other hand, heritable parasite resistance is a key assumption in Hamilton & Zuk´s theory of parasite-mediated sexual selection. To what extend *Monocystis* resistance and/or tolerance is heritable is not yet understood.

The male function might be more eager to mate with a virgin partner (Koene and Ter Maat 2005; Dillen, Jordaens et al. 2008) particularly at the beginning of the pair phase. Later on, a copulation rate that is higher than necessary to refill on allosperm can be interpreted as male-driven, which is a common pattern in most hermaphrodites (Michiels 1999; Greeff and Michiels 1999b). Michiels and Koene´s model (2006) shows that hermaphrodites will always accept higher mating costs than gonochorists. Remating to gain paternity compensates for the additional costs paid by the female function. Sex allocation theory assumes that there is a fixed energy budget that can be divided between either sexual function (Charnov 1982), so one could argue that investment in mate choice behaviour as well as benefits of the latter should be clearly assigned to a particular gender. In reciprocally mating hermaphrodites, it is however very difficult to decide which function exhibits choice. Cross-sex effects may play a considerable role because within one

body, male and female capacities will be closer interlinked than predicted by sex allocation theory (Anthes, David et al. 2010), Earthworms could adopt the rule of thumb that “good males are good females”, a condition that is often found in hermaphrodites (Schärer, Sandner et al. 2005; Anthes, Putz et al. 2006b).

Conclusion

I could show that *Monocystis* infections indeed have an effect on *L. terrestris* mate preferences: Uninfected individuals behaved differently when presented with an infected partner, suggesting that precopulatory parasite-mediated mate choice is acting in this species. I conclude that earthworms must be able to perceive parasite burden in their conspecifics, either directly or indirectly via overall condition. To understand the underlying mechanism, further experimental investigation would be necessary.

IV. MULTI-TRAIT ASSORTATIVE MATING IN A SIMULTANEOUS HERMAPHRODITE

ABSTRACT

To examine mate choice patterns in gonochorists, a specimen of the choosing gender is exposed to two or more individuals of the opposite sex. In hermaphrodites, gender roles cannot be allocated in this way, and interference or group effects may be important. The obligatory outcrossing hermaphrodite *L. terrestris* is used to study assortative mating for size, parasite infection and colouration. Earthworms were kept under video surveillance in randomly assigned groups of six for 2 months. I found that parasite infection was associated with lower sperm concentration, poorer growth and less explicit colouration, indicative of a cost of parasitism. Assortative mating was associated with no. of cocoons, colouration and sperm cell abundance, separately and in combination. In general, earthworms avoided mating over long distances and I corrected the analysis for this effect. I also describe a new behaviour of single individuals with a copulating pair. The relevance of my findings is discussed in the light of earthworm immunity and its relation with colouration and mate choice.

INTRODUCTION

Mate choice – gathering evidence in gonochoristic species

Research on the use of spectacular ornamentation of males to attract females has paved the way for general interest on how animals choose their mates. In classical mate choice experiments, a specimen of the choosing gender (usually the female) is exposed to two or more specimen of the opposite sex (male) (Ryan 1980; Collins, Hubbard et al. 1994; Gerhardt, Tanner et al. 2000; Shaw 2000). The choice opportunities that are offered differ in the trait that the researcher suspects to be under sexual selection. The trait should reliably reveal the male´s condition and could be e.g. size (Cator, Ng'Habi et al. 2010), colour patterns (Milinski and Bakker 1990) and other indicators of vigour (Verburgt, Ferreira et al. 2011) or parasite resistance (Hill 1990; Hill 1991). The behaviour of the female is observed to draw conclusions about what trait values she prefers. Although those rather simple experiments can provide valuable insight into mate choice, they often miss the bigger picture: Their outcome might depend on the trait chosen by the observer. Usually, only this particular trait is examined, leaving plenty of room for other factors to interfere (Haerty, Gentilhomme et al. 2007; Lancaster, Hipsley et al. 2009). It could be shown that females do not exclusively prefer one extreme trait value, but often choose their partners according to their own condition or set of genes to obtain the best genome-environment match for their offspring (Bos, Williams et al. 2009; Pryke, Rollins et al. 2010). In order to be able to choose a best-matching partner, the female might need to sample within a group of potential mates. As the classical experimental set up contains only two individuals to choose from, it rarely represents the whole range of trait values available to a female in nature.

Under natural conditions, most animals live in groups or populations that offer plenty of choice opportunities. Mate encounter rates, a key to mate choice evolution (Kokko and Monaghan 2001; Kokko and Johnstone 2002) increase even more when members of a species gather in one location during the mating season (Willis and Birch 1982; Warner 1995; Gibson 1996; James, Eckert et al. 2005). Quite often, these group settings are necessary to trigger a certain reproductive behaviour such as pheromone production (Krupp, Kent et al. 2008), mating calls (Bremner, Trippel et al. 2002) or spawning (Colin 2010). Behaviour that is displayed between members of one gender can be used as a choice criterion for the other gender. It is obvious that

the classic set up could hinder such behaviour because e.g. a certain group size threshold is not met.

Species that are highly variable in both sexually selected traits and (female) choice patterns may have the luxury of an abundance of basic material to evolve mate choice. One of the most variable groups of organisms are hermaphrodites, organisms that possess both male and female genitalia. The ability to divide resources flexibly to male and female function as well as arising conflicts over sexual roles are sources for high intraspecific variation (Michiels and Bakovski 2000; Anthes, Putz et al. 2005; Schärer, Sandner et al. 2005; Sprenger, Lange et al. 2009). As they unite male and female functions within one body, it is most likely that a combination of traits is more important than a single trait during the mate choice process.

Setting up hermaphrodites- a challenge

The evolution of hermaphroditic sexual traits will differ from gonochoristic ones because sex-related traits are exposed in every generation - they cannot stay hidden like male traits in gonochorists when they end up in a female (Connallon and Clark 2010). Therefore, mate choice scenarios in hermaphrodites are certainly more complicated than in gonochorists. Hermaphrodites are generally expected to act more as a male during mating. As paternal fitness is much easier to achieve, they should seize the opportunity to fertilize as many of their partners´ eggs as possible rather than trying to get their own eggs fertilized (Charnov 1979). This can lead to a conflict over the amount of sperm each partner is allowed to give (Michiels and Newman 1998). In other situations, individuals may be reluctant to donate sperm when sperm becomes costly: More sperm should be produced when allosperm digestion is as common as is true for most sperm storing hermaphroditic species (Greeff and Michiels 1999b). In cases of sperm competition, an individual will need large amounts of sperm to compete successfully for the eggs of the mate.

Solutions to such sexual conflicts can take various forms, such as gamete trading (Leonard and Lukowiak 1991; Vreys and Michiels 1998) also called conditional reciprocity (Chaine and Angeloni 2005; Schmitt, Anthes et al. 2007) or partner manipulation (Anthes and Michiels 2007). The reciprocal nature of hermaphrodite mating strategies favours the evolution of assortative mating where individuals will prefer partners that are similar to themselves in some key traits (Gregorius and

Hattemer 1987; Pal, Erlandsson et al. 2006). This process of reciprocal choice is enhanced in animals where one trait, e.g. big body size, is favoured by the majority of the population. As an evolutionary consequence, assortative mating may therefore lead to stabilizing selection of the trait involved (Vreys and Michiels 1997; Michiels and Newman 1998; Anthes, Putz et al. 2006a; Anthes, Putz et al. 2006b). Assortative mating occurs especially when potential partners employ the same choice criteria, which is particularly likely in outcrossing simultaneous hermaphrodites. Such animals act as sperm donor and receiver at the same time, and therefore have shared interests during copulation. This should promote the evolution of assortative mating. Up to now, most studies on hermaphrodite mate choice have been conducted on hermaphrodites that can choose their gender role (Holm 1979; Angeloni 2003; Chaine and Angeloni 2005; Koene, Montagne-Wajer et al. 2007; Velavan, Schulenburg et al. 2010), and only a few investigate obligatory simultaneous copulations (Peters and Michiels 1996; Vreys and Michiels 1997; Michiels, Hohner et al. 2001; Tato, Velando et al. 2006).

Lumbricus terrestris – a special hermaphrodite

The earthworm *Lumbricus terrestris L*. (Clitellata, Oligochaeta) is an outcrossing simultaneous hermaphrodite. It lives in permanent vertical burrows that usually have only one opening to the surface, making it virtually sessile when it comes to spatial distribution. Nocturnal activities take mostly place on the surface and include foraging for litter, mate search and mating. During mate search, *Lumbricus terrestris* exhibits extensive pre-copulatory reciprocal burrow visits. This behaviour can be interpreted as a kind of mate assessment or courtship (Nuutinen and Butt 1997). In the following mating, the earthworms stay anchored in their burrows with their caudal ends while the front ends are attached in a typically S-shaped position. In addition, special copulatory setae or bristles are pierced into the partners´ body for further bonding (Koene, Pförtner et al. 2005). Retraction into the burrow is thus much slower in mating pairs than in single individuals. At the end of a mating, the pair has to detach by force, often resulting in one, usually the smaller, being pulled out of its burrow. As copulations usually start between 0200 and 0400 and last about 2-6 hours, mating pairs and individuals stranded on the surface are conspicuous for predators, e.g. the proverbial early bird (Michiels, Hohner et al. 2001). Therefore, mating over long

distances with almost the whole body exposed, or with much bigger individuals, poses a serious risk to an individual.

A more subtle risk can be found within the partner's body cavity: *L. terrestris* is parasitized by the gregarine protozoan *Monocystis sp*., which lives mainly in the seminal vesicles where self-sperm matures and is stored. The parasite does not harm its host heavily, but sometimes castrates its host when parasite cells occur in such high densities, that they block the vasa deferentia (Sims and Gerard 1985). There are no indications that *Monocystis* is transmitted sexually (Field and Michiels 2006). In addition (Field and Michiels 2005) found a negative effect of *Monocystis* infection on growth and it could be shown that mating success and mate choice depend on body size in other hermaphrodites (i.e. Angeloni et al. 2003).

Hypothesis

Based on the underlying positive relationship of body size with female fecundity (Field and Michiels 2005), I predict that mate choice according to size plays a role in *Lumbricus terrestris*. I expect that in groups composed of animals with varying size, the preference of all individuals for a large partner results in size-assortative mating. In a laboratory experiment, pairs of same-sized individuals formed earlier than pairs of differently-sized individuals (Michiels, Hohner et al. 2001). Parasite abundance could provide another mate choice criterion. Therefore, I first investigate the effects of *Monocystis* infection on sperm cell abundance and growth. Then, I address the questions whether earthworms mate assortatively with respect to parasite and sperm cell abundance. If earthworms use parasite abundance as mate choice criterion, how could they perceive the strength of a *Monocystis* infection in their partner? It is known that skin colour correlates positively with parasite concentration. Although *L. terrestris* possesses light receptors in its head region, it needs to be proven to what degree it is a useful cue to determine a partner's parasite load (Field et al. 2003). If all earthworms of a group prefer partners with low parasite loads, this should result in parasite and/or colour-assortative mating. Finally, I analyse the possibilities that mate choice is best explained by a combination of the criteria mentioned above. To account for the risk of being pulled out of the home burrow by the partner at the end of copulation, distance between burrows is used as a correction factor in all assortative mating analyses. Mating behaviour and special group behaviour is recorded by using long term (42 nights) video recording of 21 groups of six

earthworms each under controlled conditions in a climate chamber. As non-infected earthworms of known age are scarce, I use a diverse natural population in a correlative approach.

MATERIALS AND METHODS

Experimental set up

Adult *L. terrestris* were purchased from a fish bait supplier. They were kept in a climate chamber at 15°C and 80% humidity with a 10:14 day/night cycle in artificial burrows made of cable ducts. These can be opened along the long side to check the worms on a regular basis. I filled them with loamy soil that had been dried, rewetted to a creamy consistency and passed through a mesh (4 x 4 mm) to remove bigger particles. This rather liquid substrate was then mixed with coconut fibre (a common terraria substrate) resulting in a humid but solid substrate. The coconut fibre prevents moisture loss and fungal growth. The filled cable ducts were closed at the bottom end with duct tape to prevent escapes. To simulate ground water levels, the duct tape was punctured and this end of the cable duct was placed into dH_2O. The worms were inserted into the soil from the top, allowing them to dig their own burrow. As worms feed on the surface around the burrow entrance, I fitted a one litre wide-mouth PE flask with a matching hole in the bottom on top of the cable duct. The bottom of the flask was then covered with substrate and food. For further details, also see (Field and Michiels 2005). Earthworms were kept in this single housing for 6 weeks to acclimatize to climate chamber conditions. Before the start of the experiment, they were weighed and placed into fresh substrate. They were then randomised by weight and transferred into groups of six worms. For this purpose, the artificial burrows were arranged in a hexagon (**Figure 1**). A total number of 126 *L. terrestris* were used to create 21 replicate hexagon groups. They were fed twice a week with frozen lettuce. Behaviour was recorded using time-lapse infrared recording from 18:30 till 05:30 for 42 days. Videos were checked daily for worm escapes, relocation into other burrows and non-moving, possibly dead earthworms. Doing so is essential to assure that the ID of each earthworm could be correctly assigned at all times. If an earthworm did not emerge from its burrow for more than two consecutive nights, its burrow was opened and checked. After 42 days the observation phase was terminated because a total of 18 (14 %) earthworms had died in 2 consecutive nights.

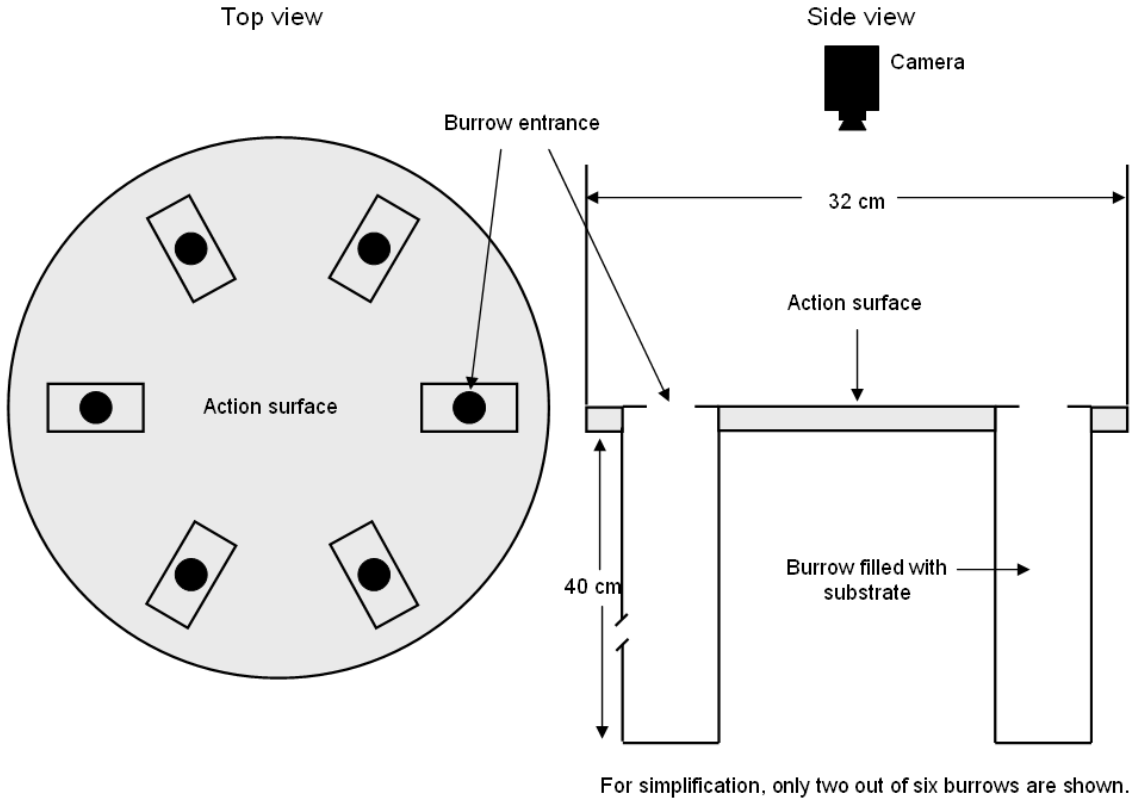

Figure 1 Scheme of one hexagonal observation unit (= Neighbourhood)

Video analysis

I recorded time and duration of mating, and partner ID. A mating starts when partners are completely attached in the typical S-shape position. I also recorded all individuals that interacted with the mating pair.

Spectral analysis

After the observation period, all worms were weighed and sedated for 3 min in sparkling water and on ice. They were photographed under standardised light conditions under a stereo microscope (Leica DFC 320 on a Leica MZ 16F) Pictures were taken from the anterior ventral region, the ventral clitellum and the ventral tail tip. Worms were cut behind the clitellum with scissors. They received a randomised number and both parts were stored in 100% Ethanol at -20°C.

Pictures were analysed using Adobe Photoshop® CS2. A non-reflecting square (10 x 10 pixels) from the middle of the target body part was selected and digitally blurred (Gaussian blur). Colour values (hue, saturation and brightness) were measured with the colour pipette tool. This was done three times for each body part and measures were subsequently averaged. Several colour traits were correlated

with each other, so a principal component (PC) analysis was performed, and the first three PCs were used for further analysis (**Table 1**, **Table 2**).

$PC_{colours}$	%	X^2	df	p
1	26.83	176.00	44	< 0.001
2	16.00	115.89	35	< 0.001

Table 1 Statistics of the Principal Component Analysis of colour measures for the first two Principal Components.

$PC1_{colours}$	Spectral trait $PC1_{colours}$	$PC2_{colours}$	Spectral trait $PC2_{colours}$
0.537	**Front B (%)**	**0.622**	**Clitellum H (°)**
0.532	**Front S (%)**	**0.567**	**Front H (°)**
0.424	**Tail B (%)**	**0.441**	**Clitellum B (%)**
0.384	Clitellum B (%)	0.251	Tail S (%)
0.214	Clitellum S (%)	0.069	Front S (%)
0.126	Tail S (%)	0.047	Tail H (°)
0.026	Tail H (°)	-0.010	Clitellum S (%)
-0.023	Clitellum H (°)	-0.085	Tail B (%)
-0.197	Front H (°)	-0.104	Front B (%)

Table 2 Eigenvalues for $PC1_{colours}$ and $PC2_{colours}$ of the Principal Component Analysis. Eigenvalues correspond to the variance in the data explained by the respective component.

In interpreting the colour combination pattern of the Principal Component Analysis, a trait measure was said to load on a component if the Eigenvector loading was 0.40 or greater for that component, and was less than 0.40 for the others (Andersson and Simmons 2006). Thus, $PC1_{colours}$ is mainly composed of tail brightness, front

saturation and front brightness, $PC2_{colour}$ is mainly composed of front hue, clitellum hue and clitellum brightness and $PC3_{colour}$ is mainly composed of tail hue.

Dissection

Worms were defrosted prior to dissection. The anterior part was opened with a scalpel. The 3 pairs of seminal vesicles were removed using eye scissors. They were placed in 1.5 ml Eppendorf cups and weighed with a precision scale (± 0.1 mg). Assuming that 1 mg = 1 µl, the double amount of Ringer solution was added, leading to a dilution of 1:3. Seminal vesicles were stored at -20°C.

Parasite & sperm count

Seminal vesicles were defrosted prior to the counting procedure. They were homogenized using a hand homogenizer mounted on an electric drill. Further homogenization was achieved by sonication with an ultrasound Sonicator (UW 2070, Bandelin electronic, setting: 4 s, cycle 4). 10 µl of this solution were diluted with 90 µl of Ringer solution, leading to a stock solution with dilution of 1:30.

The *Monocystis* spore concentration in the seminal vesicles was determined with a Thoma counting chamber (small square area 0.0025 mm², Thoma CE, Superior Marienfeld, Germany) under a phase contrast microscope (DM 5000 B, Leica) with magnitude 40 x 10. *Monocystis* spores can easily be recognized by their fusiform shape. For details see the protocol of (Field, Schirp et al. 2003). *Monocystis* counts were taken twice for each sample and then averaged.

To estimate sperm concentrations, the stock solution was vortexed for 1 min to break up sperm clumps, then diluted again 1:10, sonicated (UW 2070, Bandelin electronic, setting: 4 s, cycle 4) and vortexed again for 1 min. Sperm concentration was determined using a Thoma counting chamber (small square area 0.0025 mm², Thoma CE, Superior Marienfeld, Germany) under a phase contrast microscope (DM 5000 B, Leica) with magnitude 40 x 10. Sperm counts were taken three times for each sample and then averaged.

Statistical procedures

Three neighbourhoods were excluded from the analysis because only 3 worms survived until the end of the observation phase.

Data were transformed where necessary to obtain normal distributions (parasite concentration, sperm concentration and no. of segments). Transformed data are marked with §. To deal with missing values, dead worms were checked for non-random mortality regarding starting weight within the observation phase, as weight is a good indicator for health. The starting weight of dead worms did not differ from surviving worms (*Kruskal-Wallis-test, $X^2 = 0.16$, $df = 1$, $p = 0.69$*) and dead worms also did not copulate more or less often than their surviving neighbours during the period in which they were still alive (*Kruskal-Wallis-test, $X^2 = 2.51$, $df = 1$, $p = 0.11$*).

In order to estimate values for body weight at the end of the experiment of individuals that had died during the experiment, I used a linear regression model based on all surviving worms (*weight at end (g) = 2.92 + 0.61 x weight at start (g), Linear Regression, $R^2 = 0.34$, $F = 45.46$, $df = 1$ & 87, $p < 0.001$*). The same procedure was used to fill in missing values for vesicle weight (*vesicle weight (mg) = 24.87 + 11.43 x weight start (g), Linear Regression, $R^2 = 0.24$, $F = 27.46$, $df = 1$ & 88, $p < 0.001$*). As all other missing parameters (parasite concentration, sperm concentration, no. of segments, no. of cocoons) were not in a clear cause- and effect relationship with others to obtain a reliable estimate, these values were filled with a random normal distribution of the values of the whole population (**Table 3**). This procedure was necessary to maintain a group size of 6 individuals / neighbourhood because a balanced design is a prerequisite for the following analyses. In the final pair-wise comparison however, individuals with missing values were excluded to avoid impact of random values on final ranking.

Trait	$\bar{x}$	SD
Parasite concentration§	69564	56634
Sperm concentration§	420836	357078
No of segments+	102	87
No of cocoons	8.716	3.757

Table 3 Values for random normal distributions to fill in missing values.
§ - parameter was ln(x) transformed
+ - parameter was transformed by $x^{4.5}$
For better illustration, $\bar{x}$ and SD were retransformed in this table.

In order to test if *L. terrestris* uses a combination of traits to choose their partners, I fused correlating parameters by means of a Principal Component (PC) Analysis. The first two PCs of the main trait combination (consisting of No. of cocoons, ln(parasite concentration), ln(sperm concentration), ln(total no of sperm) and $PC1_{colours}$) were used for the assortative mating analysis (**Table 4**, **Table 5**).

$PC_{main\ parameters}$	%	X^2	df	p
1	40.44	225.14	14	< 0.001
2	24.85	182.77	9	< 0.001

Table 4 Statistics of Principal Component Analysis of main parameters.

$PC1_{main\ traits}$	Trait	$PC2_{main\ traits}$	Trait
0.682	ln(Sperm concentration)	0.661	$PC1_{colour}$
0.673	ln(Total no. of sperm)	0.490	No. of cocoons
0.117	$PC1_{colour}$	-0.162	ln(Sperm concentration)
0.062	No. of cocoons	-0.189	ln(Total no. of sperm)
-0.254	ln(Parasite concentration)	-0.511	ln(Parasite concentration)

Table 5 Eigenvectors of $PC1_{main\ traits}$ and $PC2_{main\ traits}$ sorted by Eigenvalue.

As the independent variable Neighbourhood or the interaction term between Neighbourhood and ln(parasite concentration did not show any significant effect on ln(sperm concentration) (*Standard Least Squares Model, Neighbourhood F = 0.98, df = 17, p = 0.49, Neighbourhood*ln(parasite concentration) F = 0.77, df = 17, p = 0.71*) and Neighbourhoods were not clustered graphically, data were pooled across neighbourhoods for the analysis of the effect of parasite concentration on sperm concentration (Result section, **Figure 2**). The same applied for the effect of parasite concentration on growth and cocoon production (**Figure 3**): Neighbourhood or the interaction term between Neighbourhood and ln(parasite concentration) did not show any significant effect on growth (*Standard Least Squares Modell, Neighbourhood F = 0.71, df = 17, p = 0.78, Neighbourhood*ln(parasite*

concentration) F = 1.63, df = 17, p = 0.09) and cocoon production (*Standard Least Squares Modell, Neighbourhood F = 1.09, df = 17, p = 0.39, Neighbourhood*ln(parasite concentration) F = 0.41, df = 17, p = 0.98*) and data were pooled across neighbourhoods for these analyses.

Assortative mating

To test for assortative mating, I used an individual, focal based approach. For each individual, I compared a weighted trait value of all partners each individual copulated with against the same weighted trait value of all neighbours that were rejected as a partner, once a partner had been chosen.

First, the value difference of the obtained trait measures between each earthworm in a neighbourhood was calculated in 6 x 6 table (Example for initial body weight: **Table 6**). Second, the resulting trait value differences were multiplied with the number of copulations (**Table 7**) that occurred between the respective partners to account for the differences in no. of copulations (Result: **Table 9**). In a second table, the value differences were multiplied with "no of non-copulation of partner A" = (total no of copulations of partner A – no. of copulation between A and B) / 4) (**Table 8**) to obtain a corresponding value for all cases were a worm was not chosen (**Table 10**).

worm ID		A1	A2	A3	A4	A5	A6
	weight start (g)	5.79	6.37	7.3	5.63	6.01	7.51
A1	5.79		0.58	1.51	0.16	0.22	1.72
A2	6.37	0.58		0.93	0.74	0.36	1.14
A3	7.3	1.51	0.93		1.67	1.29	0.21
A4	5.63	0.16	0.74	1.67		0.38	1.88
A5	6.01	0.22	0.36	1.29	0.38		1.5
A6	7.51	1.72	1.14	0.21	1.88	1.5	

Table 6 Example table - Calculation of value differences.

no of copulations	A1	A2	A3	A4	A5	A6
A1		1	1	1	0	1
A2	1		0	3	0	0
A3	1	0		2	2	0
A4	1	3	2		0	1
A5	0	0	2	0		2
A6	1	1	0	0	2	

Table 7 Example table – no. of copulations**.**

no of non-copulations	A1	A2	A3	A4	A5	A6
A1		0.75	0.75	0.75	1.00	0.75
A2	0.75		1.00	0.25	1.00	1.00
A3	1.00	1.25		0.75	0.75	1.25
A4	1.50	1.00	1.25		1.75	1.50
A5	1.00	1.00	0.50	1.00		0.50
A6	0.75	0.75	1.00	1.00	0.50	

Table 8 Example table – no. of non- copulations**.**

Weight difference x no of copulations	A1	A2	A3	A4	A5	A6
A1		0.58	1.51	0.16	0	1.72
A2	0.58		0	2.22	0	0
A3	1.51	0		3.34	2.58	0
A4	0.16	2.22	3.34		0	1.88
A5	0	0	2.58	0		3
A6	1.72	1.14	0	0	3	

Table 9 Example table – correction for no of copulations.

Weight difference x no of non-copulations	A1	A2	A3	A4	A5	A6
A1	0	0.44	1.13	0.12	0.22	1.29
A2	0.44	0	0.93	0.185	0.36	1.14
A3	1.51	1.16	0	1.25	0.97	0.26
A4	0.24	0.74	2.09	0	0.67	2.82
A5	0.22	0.36	0.65	0.38	0	0.75
A6	1.29	0.86	0.21	1.88	0.75	0

Table 10 Example table – correction for no of non-copulations**.**

In a third step, I corrected for different distances between the potential partners in the hexagon as distance might play a role in mate choice (Michiels, Hohner et al. 2001). I decided to take the reverse observed frequencies of short, middle and long distance matings as correction factors (**Table 11**, see also Results **Figure 5**). Partners further away should get a higher impact on the trait difference value, while partners close by could not be chosen because of their trait values but for their close proximity to the focal´s burrow.

Correction factor for distance	A1	A2	A3	A4	A5	A6
A1		0.23	0.34	0.43	0.34	0.23
A2	0.23		0.23	0.34	0.43	0.34
A3	0.34	0.23		0.23	0.34	0.43
A4	0.48	0.34	0.23		0.23	0.34
A5	0.34	0.43	0.34	0.23		0.23
A6	0.23	0.34	0.43	0.34	0.23	

Table 11 Correction factors for distance.

For each individual, I summed the resulting corrected trait value differences of all copulated and non-copulated neighbours, allowing for a pairwise comparison. If assortative mating occurs, the corrected value differences are expected to be smaller for the copulated than for the non-copulated neighbours. To obtain a more detailed picture, individuals were ranked by their own trait measurement value, and all earthworms with the same rank number were compared over the 20 neighbourhoods, giving 6 individual test ranks. Earthworms with missing values were excluded for these final tests (Example: **Table 12)**. To correct for multiple testing, a Holm-Bonferroni adjustment of p-values was applied (Holm 1979).

Individual weight (g)	Rank number = Test Run number	weight difference x no of copulations x correction factor for distance	A1	A2	A3	A4	A5	A6	**total value for copulated**	weight difference x no of non-copulations x correction factor for distance	A1	A2	A3	A4	A5	A6	**total value for not copulated**
5.79	**2**	A1		0.14	0.51	0.07	0	0.40	**1.12**	A1		0.10	0.39	0.05	0.07	0.30	**0.91**
6.37	**4**	A2	0.14		0	0.75	0	0.39	**1.28**	A2	0.14		0.27	0.13	0.19	0.39	**1.12**
7.3	**5**	A3	0.51	0		0.78	0.88	0	**2.17**	A3	0.51	0.27		0.29	0.33	0.11	**1.51**
5.63	**1**	A4	0.07	0.75	0.78		0	0.64	**2.24**	A4	0.10	0.25	0.49		0.15	0.96	**1.95**
6.01	**3**	A5	0	0	0.88	0		0.70	**1.58**	A5	0.07	0.15	0.22	0.09		0.17	**0.70**
7.51	**6**	A6	0.40	0.39	0	0.64	0.70		**2.13**	A6	0.40	0.39	0.11	0.64	0.27		**1.81**

Table 12 Example table: Final calculation of corrected trait value differences and pairwise comparison between total value for copulated and total value for not copulated.

To test whether earthworms use a combination of criteria for assortative mating, a principal component analysis (PCA) was performed with all relevant trait measures. A trait measurement was considered as relevant when it scored at least once with a p-value < 0.10 in the individual analysis. The chosen parameters were: No. of cocoons, ln(parasite concentration), ln(sperm concentration), ln(total no of sperm) and $PC1_{colours}$. The combined principal components ($PC1_{main\ traits}$ and $PC2_{main\ traits}$) were subsequently introduced into the assortative mating analysis as described above. After Holm-Bonferroni correction for multiple testing (Holm 1979), p-values below 0.05 were considered significant, and p-values with $0.05 < p < 0.10$ were considered as trends.

RESULTS

Costs of Parasitism: Effect on sperm concentration

Ln(sperm concentration) decreased with increasing ln(parasite concentration) (**Figure 2**). This effect suggests that high infection loads come at the expense of sperm production.

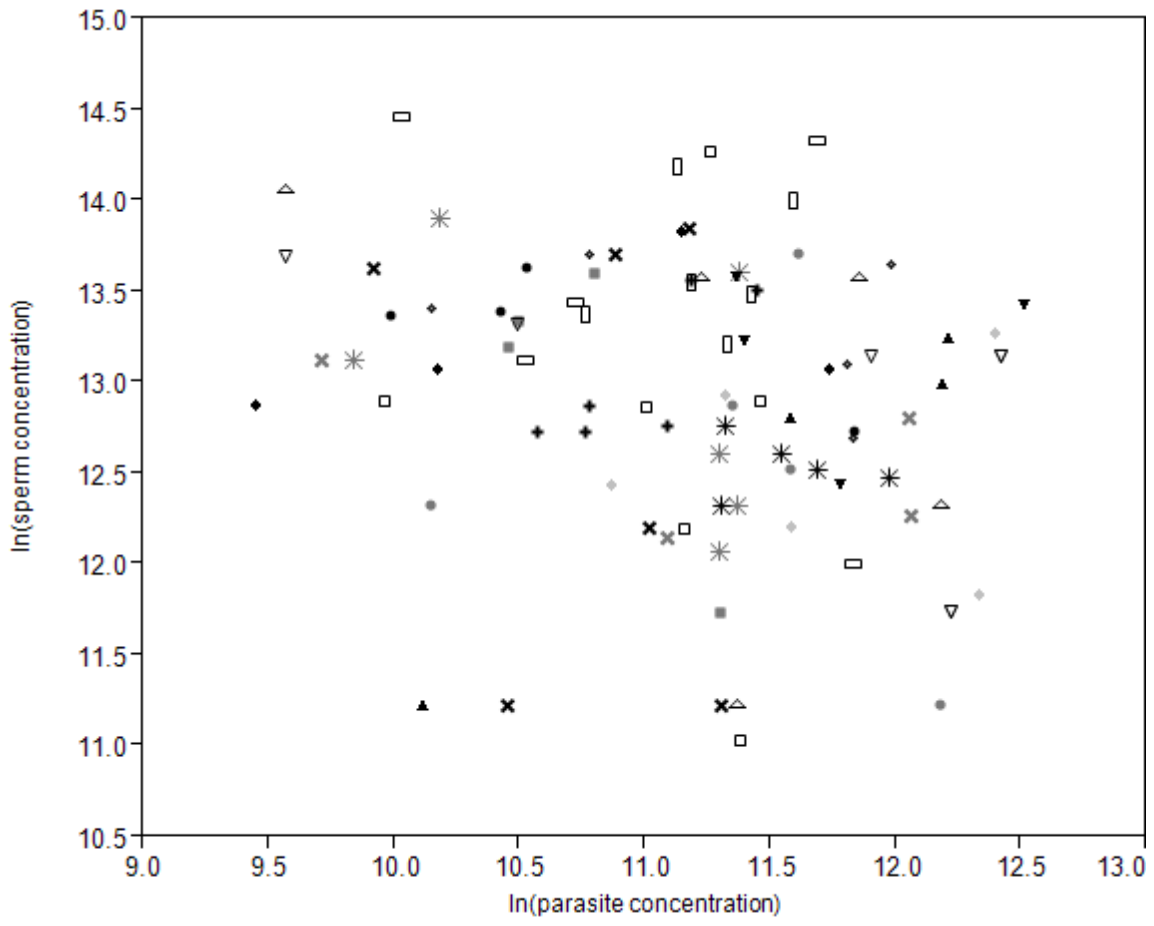

Figure 2 Correlation between ln(parasite concentration) and ln(sperm concentration). Different markers represent different Neighbourhoods (*Pearson correlation, r = - 0.22, n = 89, p = 0.041**)

Costs of parasitism: Effect on growth

Increasing ln(parasite concentration) decreased growth (**Figure 3**), but not cocoon production (*Pearson correlation, r = -0.08, n = 90, p = 0.48*), a hint that *Monocystis* infection has an overall systemic effect, but does not affect the female function directly.

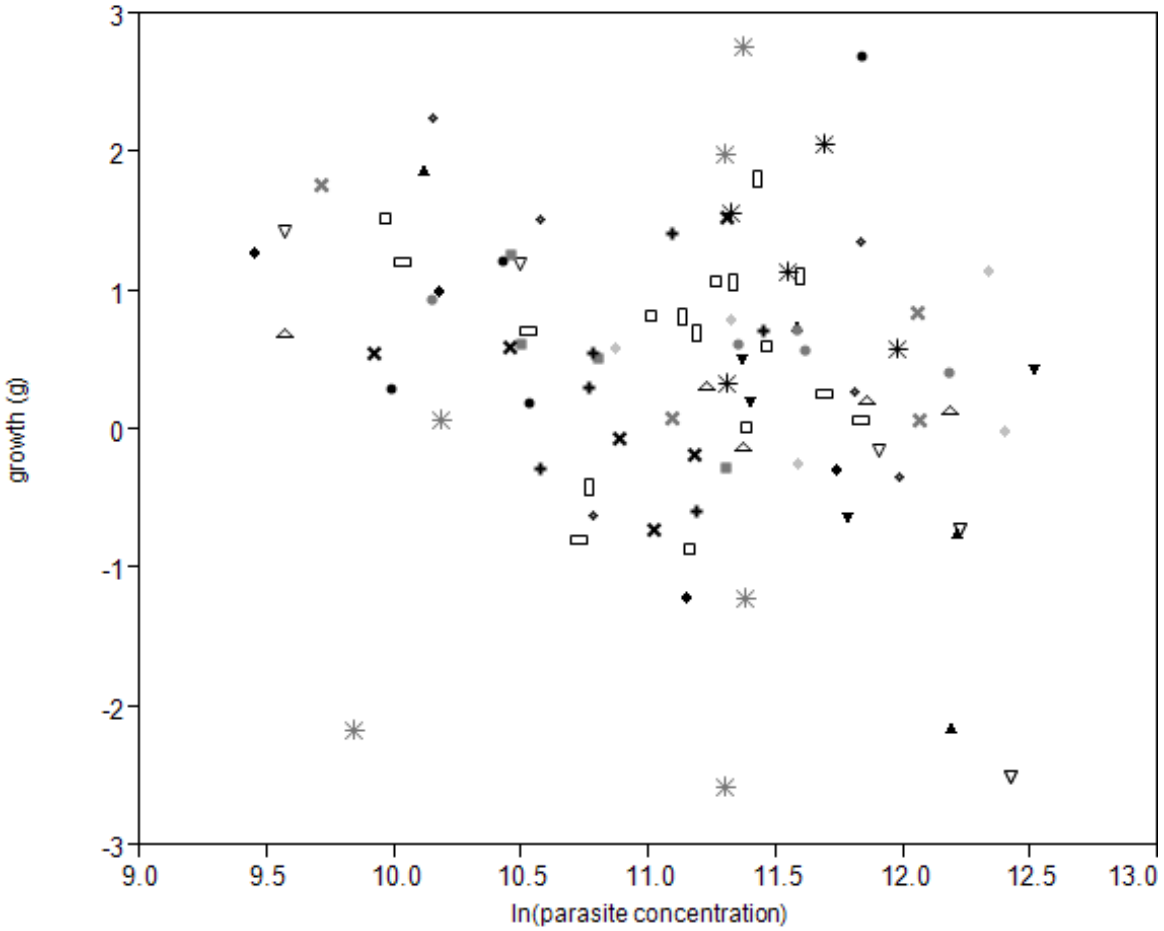

Figure 3 Correlation between ln(parasite concentration) and growth (g). Different markers represent different Neighbourhoods. (*Spearman correlation*, *rho = -0.25, n = 90, p = 0.017**)

Parasite effects on spectral traits

As Neighbourhood or the interaction term between Neighbourhood and ln(parasite concentration) did not show any significant effect on $PC1_{colours}$ (*Standard Least Squares Model, Neighbourhood F = 0.99, df = 17, p = 0.48, Neighbourhood*ln(parasite concentration) F = 1.49, df = 17, p = 0.13*) data were pooled across neighbourhoods for the following analysis. $PC1_{colours}$ was negatively correlated with ln(parasite concentration) (**Figure 4**).

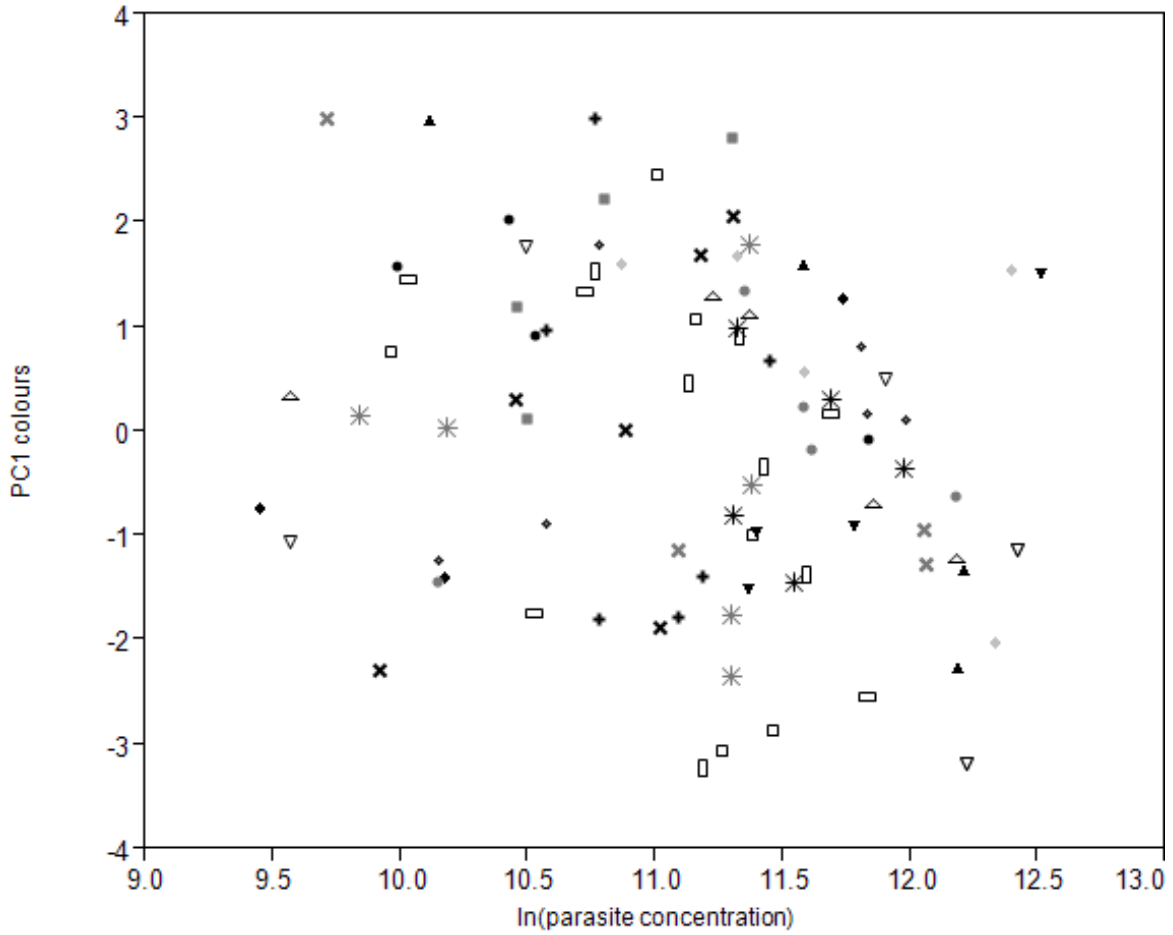

Figure 4 Correlation between ln(parasite concentration) and $PC1_{colours}$. Different markers represent different Neighbourhoods (*Pearson correlation, r = -0.22, n = 89, p = 0.039**).

Mating distances - Minimizing the pull out risk

I expected that earthworms would prefer to mate across short distances, and copulations would become rarer with increasing burrow distances. Indeed, the overall mating distances differed from the expected random distribution of 2:2:1 for short, medium and long-range copulations (**Figure 5**). This expected distribution is based on the simple fact that in a hexagonal set-up, there are two neighbours each for the short and medium distance, but only one for the long distance category on the opposite side of the arena. The observed frequencies of short, middle and long distance matings were used in the analysis of assortative mating as correction factors to disentangle distance effects from physical quality effects.

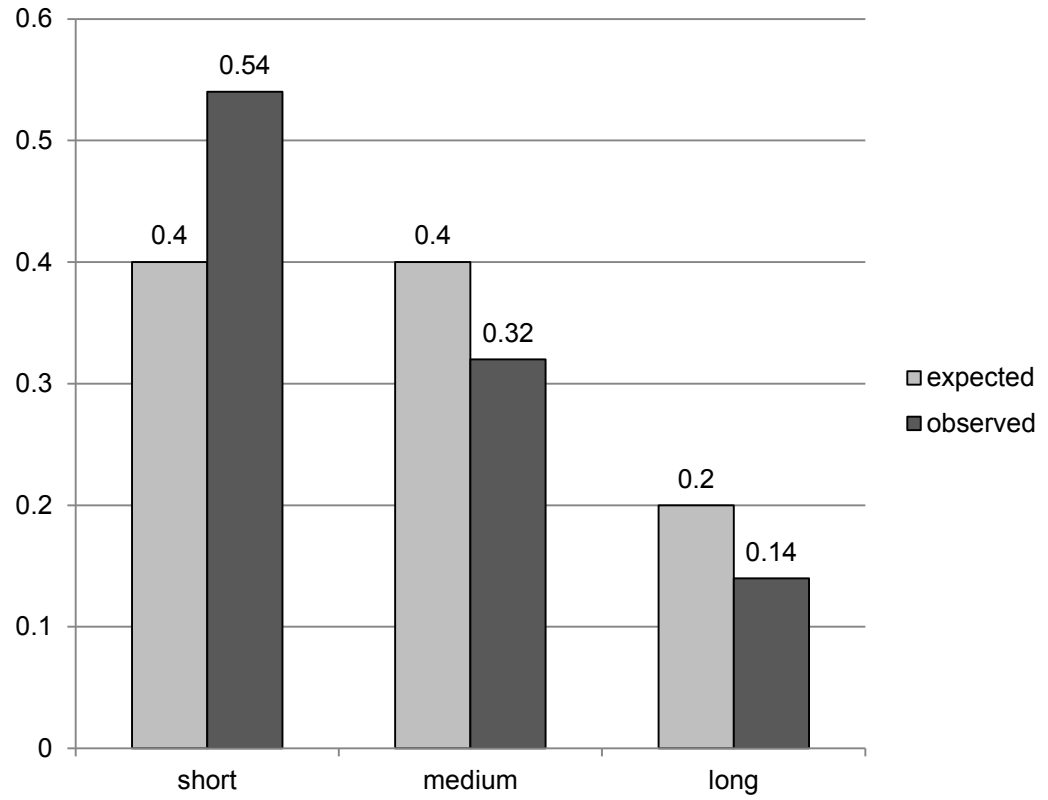

Figure 5 Expected and observed frequencies for short, medium and long range copulations. *(Likelihood Ratio test with combined p-values of each neighbourhood, t = 93.33, p < 0.001*)

Assortative mating

Results of the pairwise comparison between copulated and non-copulated partners revealed that some earthworms mated assortatively, but most did not. In detail, I found assortative mating for total number of sperm cells in test rank no. 6: As test rank numbers were assigned according to ascending trait values, this means that earthworms that have a lot of sperm cells themselves mate more often with earthworms that also have high sperm cell numbers. A similar result could be found for $PC1_{colours}$ in test rank no. 2. Here, earthworms with a low $PC1_{colours}$ value mated with similarly coloured individuals, a trend that was also noted for the neighbouring test rank numbers 1 and 3. **Figure 6**, **Figure 7** and **Figure 8** show the p-values for each test rank and trait, $p < 0.05$ is marked with o, $0.05 < p < 0.1$ with ×.

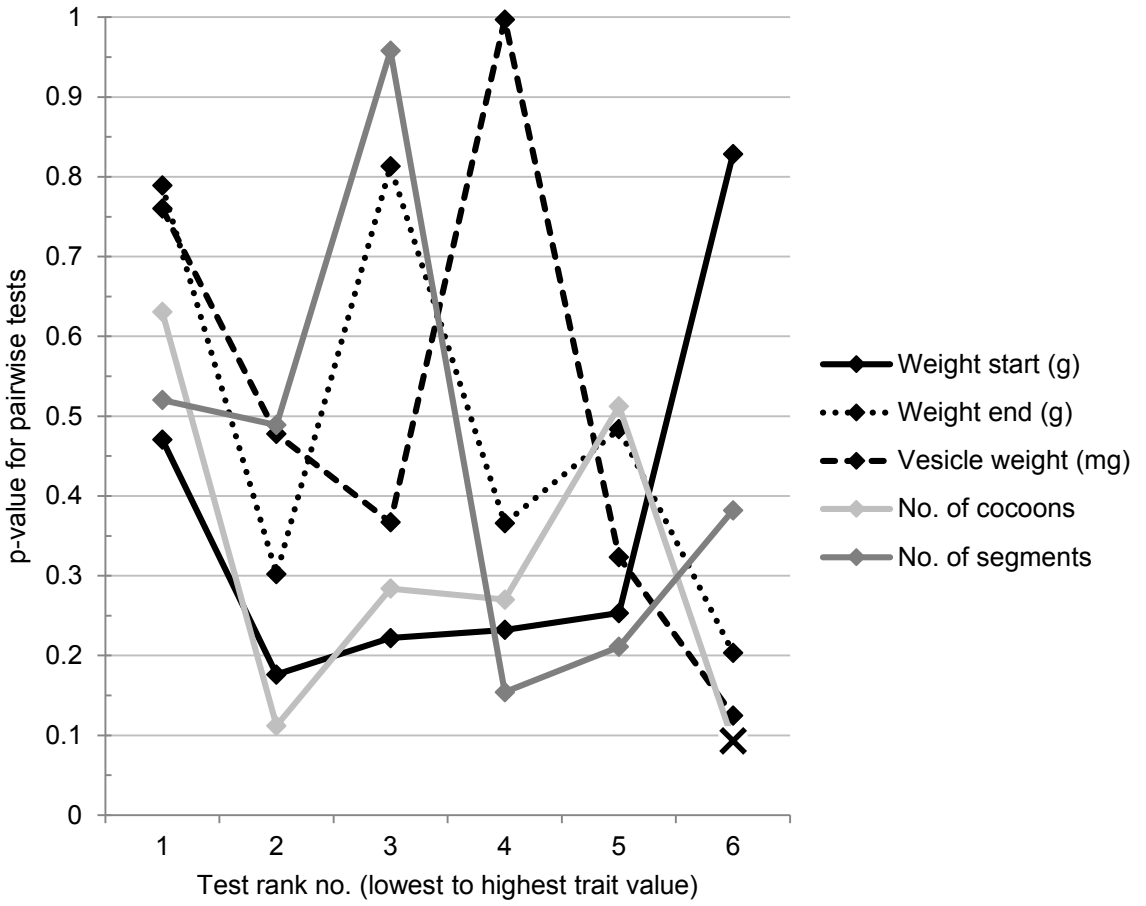

Figure 6 P-values for pairwise tests of all test rank no for body parameters.

Note that p-values tend to be lowest for extreme low (test rank 1 and 2) and high (test rank 6) trait values. For detailed statistics, see Appendix of this chapter, **Table 13** and **Table 14**.

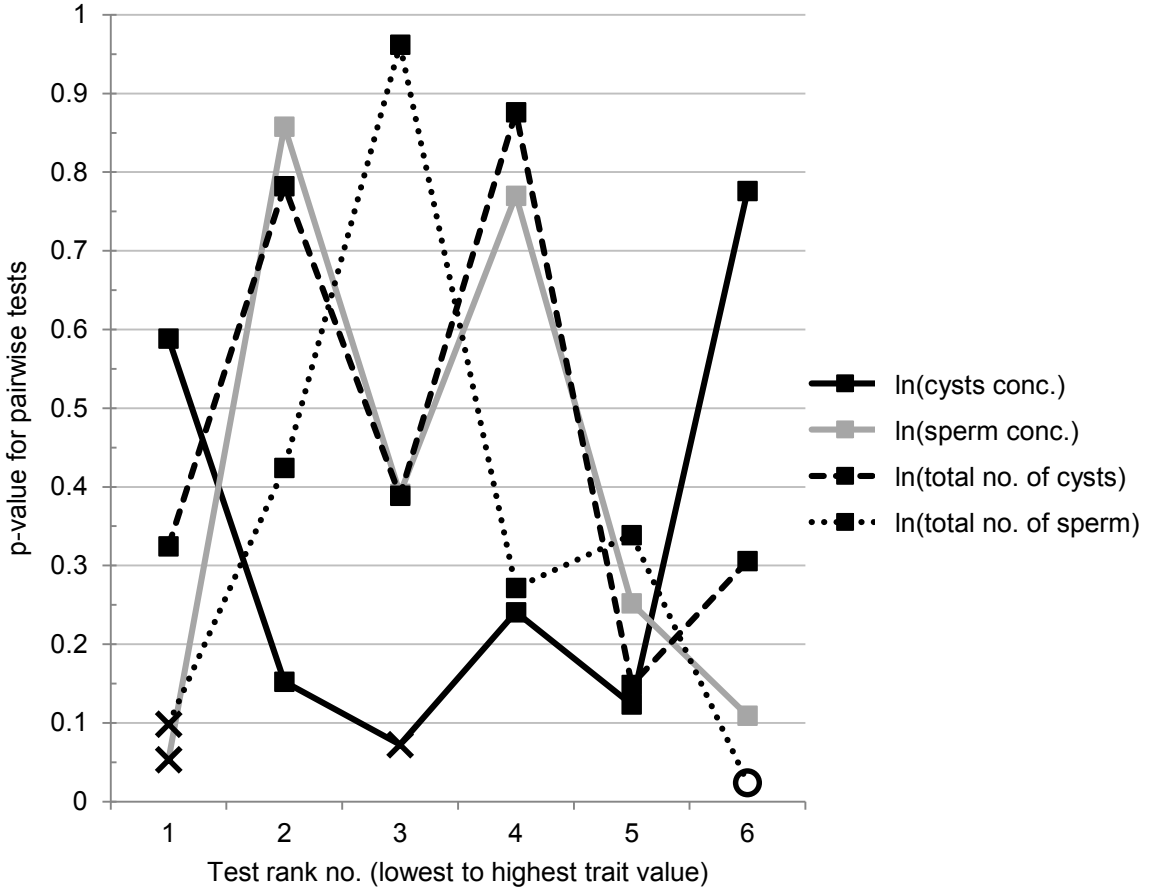

Figure 7 P-values for pairwise tests of all test rank no for sperm and parasite parameters.

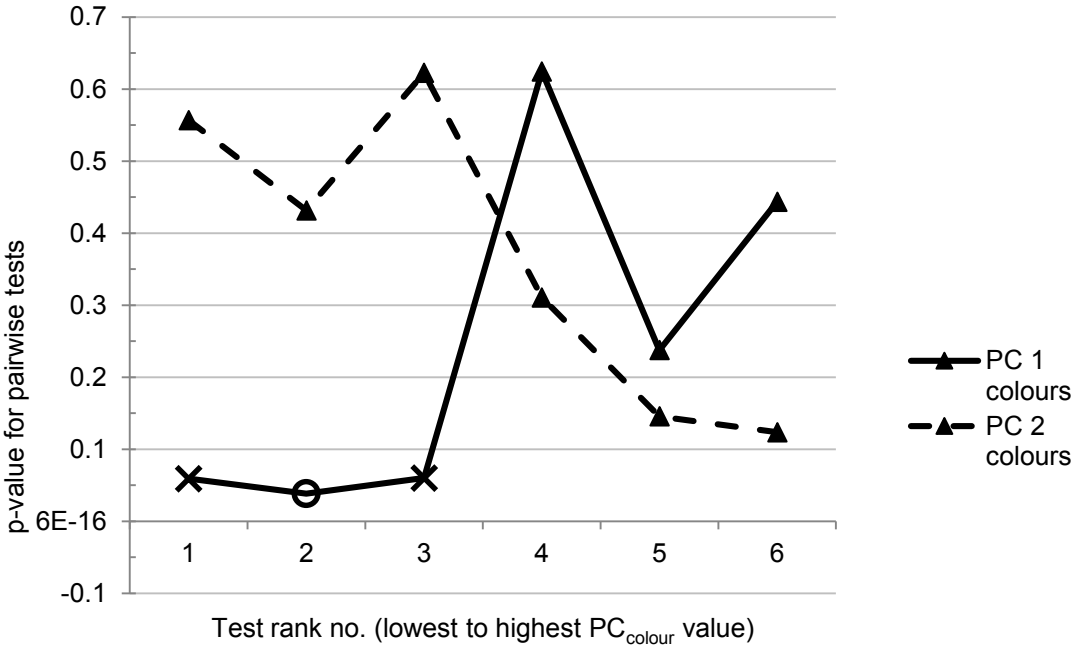

Figure 8 P-values for pairwise tests of all test rank no for colouration.

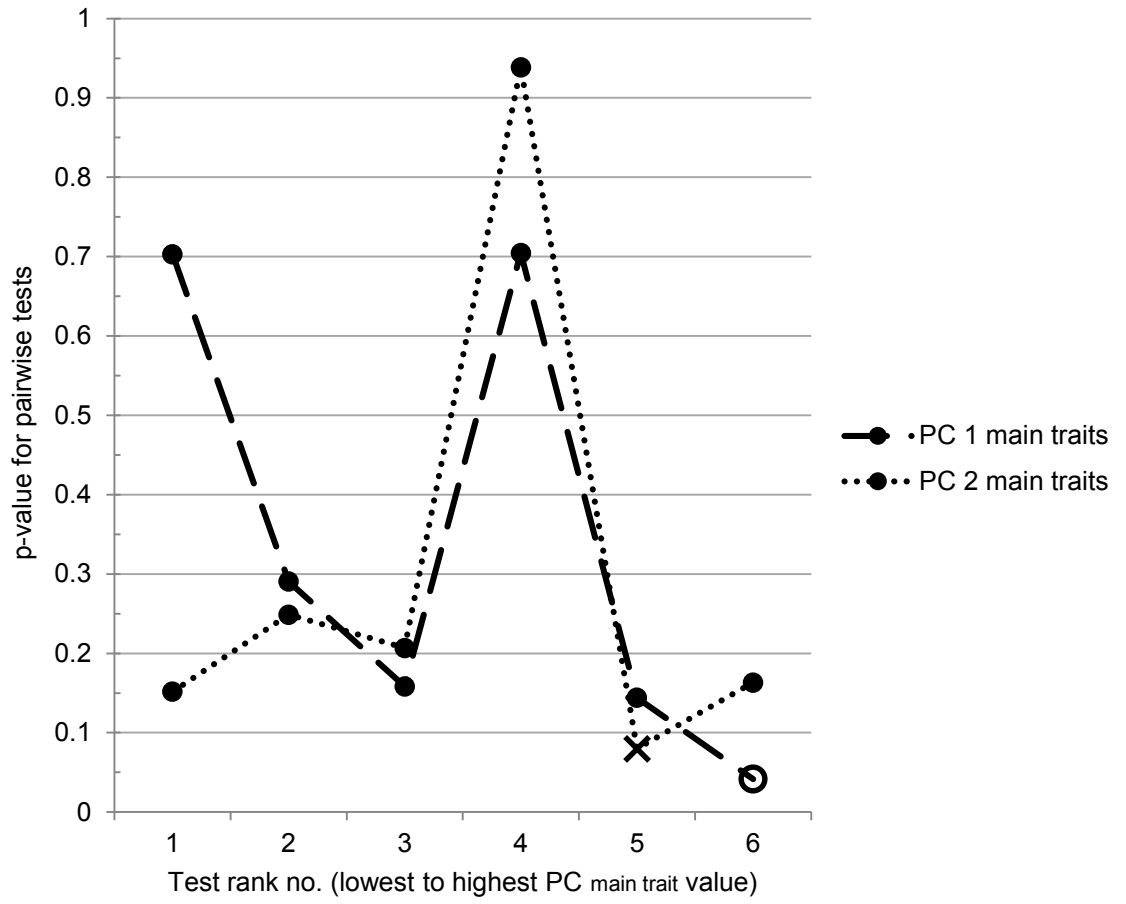

Figure 9 P-values for pairwise tests of all test rank no. for PC $_{\text{main traits}}$.

For PC1$_{\text{main traits}}$, assortative mating could be shown for test rank no. 6 and a trend for PC2$_{\text{main traits}}$ in test rank no. 5. Even for a combination of several choice factors, assortative mating could only be shown for extreme values. P-values are shown in **Figure 9** P-values for pairwise tests of all test rank no. for PC $_{\text{main traits}}$., for further details on statistics see Appendix **Table 15**.

Group behaviour

I observed food stealing from other burrows, but also novel aspects of mating behaviour. The hexagonal set-up provided more space for a mating pair than the usual pair set-up (e.g. in (Field, Schirp et al. 2003) and Chapter III). I could observe that copulations do not necessarily take place directly between the burrows, but also in other angles. The most surprising behaviour was the persistent interaction by other worms with mating pairs. One or more earthworms moved their anterior body parts, and especially the mouth region alongside or around the attached “S-shaped” anteriors of a mating couple (**Figure 11**).

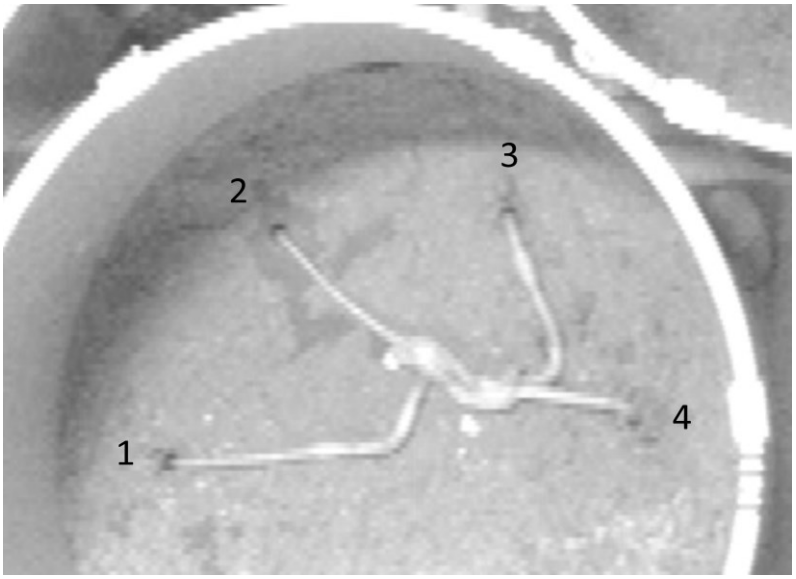

Figure 11 Infrared camera snapshot of part of a hexagonal neighbourhood set-up. Numbers indicate earthworm burrow entrances. The anterior body of their respective inhabitants can be seen on the surface.

Individual 2 & 4 are engaged in mating, individual 1 and 3 are "scanning" alongside the mating couple. The distribution of mating duration revealed a bimodal curve (smaller peak $\mu^1 = 41$ min, $\sigma^1 = 23$ min; larger peak $\mu^2 = 165$ min, $\sigma^2 = 16$ min) (**Figure** 12). I therefore only consider copulations longer than $\mu^1 + \sigma^1 = 64$ min as true matings in my analysis. Note that some copulations started late in the night and were terminated by the onset of the light phase, when infrared video observation was no longer possible. In 43% of the completed copulations, the mating couple parted while another "interacting" individual was still in contact with their anteriors. In 355 observed copulations, 67 % (238) were investigated by other individuals. Most couples were in contact with one or two individuals. Mating duration was not affected by "Neighbourhood", and the no. of interacting individuals had no significant effect on mating duration in all completely observed

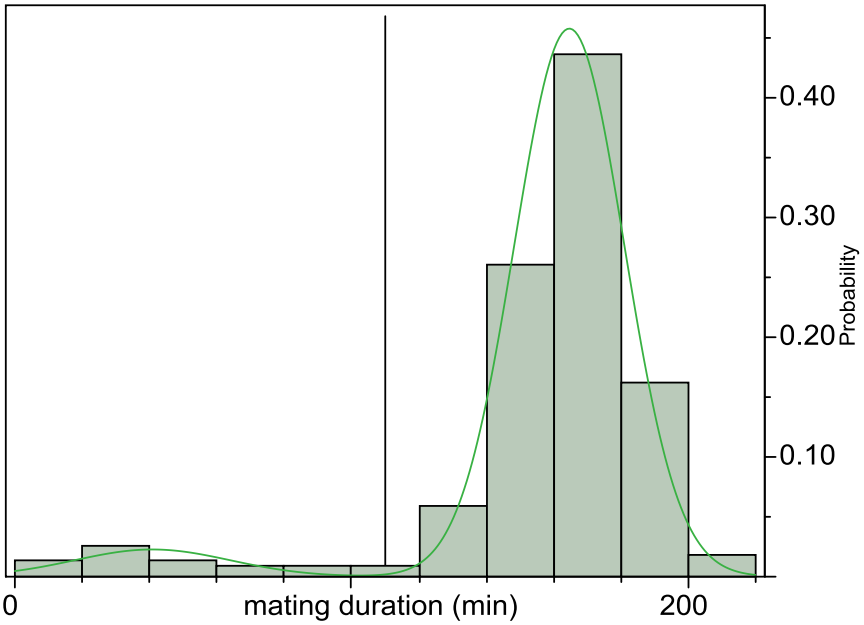

Figure 10 The distribution of mating duration for all completed matings shows a bimodal curve. Only matings falling into the higher peak on the left are considered in the analysis.

copulations (*Nested ANOVA, Neighbourhood[no of interacting individuals], df = 72, F = 1.30, p = 0.08, No. of interacting individuals, df = 4, F = 0.32, p = 0.86 overall n = 262*). However, the interacting behaviour did not affect mating duration (*Nested ANOVA, Neighbourhood[Mating ended while in contact], df = 40, F = 1.47, p = 0.045, Mating ended while in contact, df = 1, F = 2.77, p = 0.098 overall n = 262*). Note that "Neighbourhood" had an effect on mating duration.

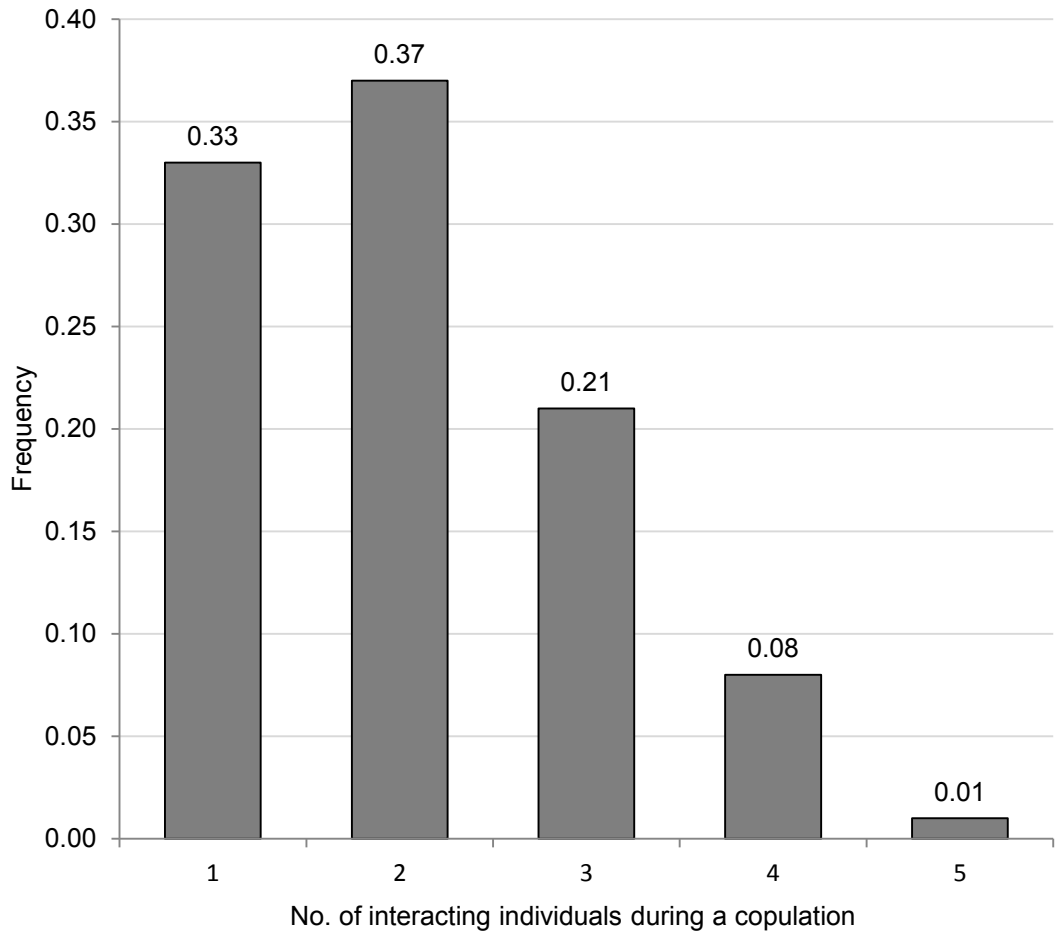

Figure 12 Frequencies of no. of interacting individuals during a copulation, n = 355.

DISCUSSION

My study revealed assortative mating in earthworms with extreme trait values for total number of sperm and colour, but no overall pattern for size- or parasite-assortative mating. When combining the most relevant mate choice parameters, only earthworms with an extremely high combination value exhibit assortative mating. I show that *Monocystis* concentration has a negative effect of sperm cell abundance and growth, and that *Monocystis* concentration affects the outer appearance of the earthworms. I also describe a novel behaviour of single worm interaction with copulating pairs.

Assortative mating

Some earthworms mated assortatively by ln(number of sperm) (Test rank no. 1 and 6) and $PC1_{colours}$ (Test rank no. 1 and 2). Interestingly, assortative mating is more articulated in cases where the focal´s parameter value is either extremely high or extremely low. Individuals with low amounts of sperm ended up mating with partners with low amounts of sperm, and individuals with high amounts of sperm mated with partners who also had high amounts of sperm in their seminal vesicles. *L. terrestris* seems to be capable of assessing the amount of sperm that the partner will be able to donate, but the physiological mechanisms remain unknown. Velando, Eiroa et al. (2008) could show that earthworms boost their ejaculate when encountering virgin partners – a first hint that the adjustment of sperm amounts according to different partner settings is possible. Sperm cell abundance is negatively affected by *Monocystis* infection, but to what extent earthworms could use the amount of sperm present in a partner as an indirect way to assess overall fitness or immunity cannot be disentangled with this data.

Assortative mating by colour occurred only in test rank no. 1 and 2, which means that individuals with a low $PC1_{colour}$ value mated preferably with partners who also have a low $PC1_{colour}$ value. A low $PC1_{colour}$ value indicates that those worms have a low front saturation and brightness, resulting in an overall darker appearance of the head region. Interestingly, the head region is used in long mate assessment behaviour prior to copulation (Nuutinen and Butt 1997) and it is known that earthworms have highly sensitive photoreceptors in their anterior region (Hess 1925). Combining these

facts with the negative relationship between $PC1_{colour}$ and ln(parasite concentration), it might well be possible that earthworms use spectral cues of the head region to assess the partners individual parasite load as one component of individual fitness, and that my observation set-up revealed only the extreme cases of very dark coloured individuals.

Costs of Parasitism

To my knowledge, this is the first study that investigates immediate effects of *Monocystis* on sperm cell abundance within the seminal vesicles. The concentration of *Monocystis* spores clearly has a negative effect on sperm cell concentration, but the underlying mechanisms remain unclear. It is quite unlikely that *Monocystis* directly feeds on sperm cells, but it is possible that *Monocystis* takes up energy and nutrition during spore formation, and that these nutrients are then lacking for sperm cell formation. When comparing the development of *Monocystis* infections to the well-known life cycle of another, related apicomplexan parasite, *Plasmodium* (the causative agent of Malaria), this scenario becomes even more plausible: The ookinetes of *Plasmodium* develop into a static oocyst in the extracellular space between the basal lamina and the basement cell membrane in the Mosquitoes salivary glands, and their chief source of nutrients is the haemolymph in which the oocyst develops until the sporozoites bud off (Puurtinen, Ketola et al. 2009). This process could be seen as equivalent to *Monocystis* sporocyst formation within the seminal vesicles of *Lumbricus terrestris*, where oocysts would also find an extracellular source of host nutrition.

Another indicator for a lack of nutrition caused by parasite infection is the negative relationship between parasite concentration and overall earthworm growth during the experiment, whereby low and moderate infected individuals tend to gain weight and highly infected individuals tend to lose weight (**Figure 3**). These results confirm the findings of (Field and Michiels 2005), where *Monocystis* concentration also had a negative effect on growth. Although both studies differ in time frame (240 vs. 42 days) and set-up, the resulting relationships between *Monocystis* concentration and growth are remarkably similar (Field and Michiels 2005): $\bar{x}_{growth/day} = 0.0094$ g, $\bar{x}_{log\ parasite\ conc.} = 7.29 \times 10^6$, $r = -0.24$, this study: $\bar{x}_{growth/day} = 0.01$ g, $\bar{x}_{log\ parasite\ conc.} = 8.87 \times 10^4$, $r = -0.21$) and suggest a constant increase in parasite concentration over time.

Colour, parasitism and mate choice

The PC colour analysis revealed that there is a negative relationship between parasite concentration and a certain combination of colour parameters, $PC1_{colours}$, which consists mainly of tail brightness, front saturation and front brightness. Considering that *Monocystis* spores are usually found in the ventral anterior body parts of *Lumbricus terrestris*, one could assume that most immune reactions against parasitic cells would occur there. A common immune reaction in invertebrates is encapsulation via melanisation, which in earthworms leads to the formation of "brown bodies" (Valembois, Lassegues et al. 1992) that are usually transported to the tail end where they accumulate and induce the autotomy of hindmost segment. These brown bodies can be regularly found in and around the seminal vesicles and are heavily melanised and even budded off the main organ, usually close to the outer epidermis (personal observations). Although (Field, Kurtz et al. 2004) could not find a relationship between immuno-active cells and parasite concentrations, the colour analysis suggests that parasites have an influence on the outer appearance of earthworms: A highly infected individual would have more melanin close to the epidermis, which would make the colour less vivid (lower saturation) and absorb more light (lower brightness). This effect would be even stronger in the lightly-rosé coloured tail tip, when brown bodies are moved there. These results are in line with the findings of (Field, Schirp et al. 2003), where front and clitellum hue were positively correlated with parasite concentration, also making highly infected individuals less explicit in their colouration.

Do earthworms combine traits to choose their partner?

When combining the most relevant parameters, only individuals with the highest value (test rank no. 6) for $PC1_{main\ parameters}$ mated assortatively. Again, this could be a result of my rather indirect way to assess assortative mating patterns, but it shows that *L. terrestris* possibly uses more than one parameter to choose its mates, as is known for many gonochoristic species such as guppies (Brooks and Endler 2001), swordfish (Hankison and Morris 2003) birds (Møller and Pomiankowski 1993; Birkhead, Fletcher et al. 1998; Pryke, Andersson et al. 2001) and humans (Thornhill and Gangestad 2006). Two alternative mechanisms for multi-parameter signalling have been proposed: The multi-message hypothesis (Johnstone 1996) states that each cue gives information on a single property thus building a mosaic of information

for the signal receiver. In contrast, a cue could only be partially reflecting the actual condition, and part of it could be redundant (redundant signal hypothesis) (Møller and Pomiankowski 1993). Considering that *L. terrestris* is a long-lived hermaphrodite with limited visual, but good chemical and tactile perception, both hypotheses are equally likely. Cues that are affected by changes in condition on long-term scales e.g. colour (e.g. through accumulation of brown bodies) or size, support the multi-message hypothesis because they honestly reflect the overall body condition. Chemical cues in the mucus (e.g. stress, cocoon production readiness) could change within the hour and might therefore be partially redundant, supporting the redundant signal hypothesis. Although in my set-up, earthworms were presented with 5 different potential partners, it might well be that those partners were not different enough to give clear results for a combined trait - assortative mating pattern.

Group behaviour

The group set up provides the first quantitative assessment of a new aspect of *L. terrestris* mating behaviour: The interaction of non-mating conspecifics with a copulating pair. This behaviour turns out to be frequent, but it does not hinder or terminate copulation earlier, in contrast to e.g. the freshwater planarian *Dugesia gonocephala* where partners separated earlier due to disturbance by conspecifics (Vreys, Schockaert et al. 1997). A possible scenario in *L. terrestris* would be the uptake of sperm by the non-copulating individuals. Sperm is transported in outer sperm grooves and accumulates as large white drops at the clitellum of each partner. They could represent an easy-to-achieve protein source for the worms. From an evolutionary point of view, feeding on sperm could also be beneficial: As *L. terrestris* is almost sessile, it is most likely that direct neighbours will be encountered during later copulations and more offspring could be fathered when fewer sperm was transferred earlier.

The interacting behaviour also sheds new light on a body structure special to earthworms: Copulatory setae that are pierced into the partners skin during copulation. The piercing enhances sperm uptake by the partner (Koene, Sundermann et al. 2002).To what extend the tight bonding plays a role in interactions between couples and other individuals still needs further investigation.

Mating distance

My results clearly indicate that *L. terrestris* prefers to mate over short distances. This could be seen as a risk-avoiding strategy: Long-range copulations may result in one partner being pulled out of its burrow and eventually being eaten by a predator. These results are in accordance with a field study (Michiels, Hohner et al. 2001) where the proportion of surfaced individuals increased with increasing mating distance. With mating distances being of such importance, I adjusted the analyses for assortative mating such that long-range matings are more meaningful in mate choice processes.

Conclusion

The group set up allowed the investigation of mating patterns in *L. terrestris*. In general, I could detect assortative mating in cases where the focal individual had extreme values for colour and sperm cell concentration. I show for the first time that sperm cell abundance is negatively affected by *Monocystis.* To what extend these two effects play a role in *L. terrestris* mate choice requires further investigation, ideally with controlled infection.

APPENDIX

Test variable	ln(parasite concentration)			ln(sperm concentration)			ln(total no of parasites)			ln(total no of sperm)			PC 1_{colors}			PC 2_{colors}		
Test run	n	t	p	n	t	p	n	t	p	n	t	p	n	t	p	n	t	p
1	16	0.5528	0.5885	15	2.1088	0.0535^{x}	16	1.0182	0.3247	15	2.7245	0.0984$^{\#x}$	15	2.982	0.0594$^{\#x}$	16	0.6008	0.5569
2	15	1.5135	0.1524	15	0.1822	0.858	15	-0.2818	0.7822	14	-0.8252	0.4242	14	2.303	0.0385$^{\#*}$	15	0.8098	0.4316
3	17	1.921	0.0727^{x}	15	0.8846	0.3913	18	0.8847	0.3887	16	0.0485	0.962	15	2.047	0.0599^{x}	12	0.5069	0.6223
4	14	1.2289	0.2409	17	0.2971	0.7702	16	0.1586	0.8761	16	1.1408	0.2718	11	0.505	0.6244	14	-1.0551	0.3106
5	14	1.6468	0.1235	13	1.2034	0.252	12	1.554	0.1485	14	0.9926	0.339	15	-1.23	0.2375	14	1.548	0.1456
6	15	0.2899	0.7762	16	1.7028	0.1092	14	1.0657	0.3059	16	2.5218	0.0235$^{\#*}$	17	0.786	0.4436	16	2.5866	0.1236$^{\#}$

Table 13 Detailed statistics on assortative mating analysis –internal parameters
$^{\#}$- Holm-Bonferroni corrected
*- significant, ^{x} - trend

Test variable	Weight start (g)			Weight end (g)			Vesicle weight (mg)			No. of cocoons			No. of segments		
Test run	n	t	p	n	t	p	n	t	p	n	t	p	n	t	p
1	21	-0.7355	0.4706	17	-0.272	0.7891	16	0.3106	0.7604	14	0.4923	0.6307	18	0.6567	0.5202
2	21	-1.4022	0.1762	14	-1.0751	0.3019	16	-0.7282	0.4777	17	1.6822	0.1119	13	-0.7138	0.489
3	21	-1.2606	0.2219	15	-0.2408	0.8132	15	0.9323	0.367	14	1.1175	0.284	13	-0.0538	0.958
4	21	1.2322	0.2322	14	0.937	0.3658	12	0.0037	0.9971	16	1.1453	0.27	16	2.476	0.1542$^{\#}$
5	21	-1.1767	0.2532	13	-0.7225	0.4838	17	1.019	0.3234	17	0.6698	0.5125	15	1.3111	0.2109
6	21	0.2198	0.8283	18	1.3227	0.2034	15	2.6048	0.1248$^{\#}$	13	1.8232	0.0933^{x}	16	-0.9005	0.3821

Table 14 Detailed statistics on assortative mating analysis – body parameters
$^{\#}$- Holm-Bonferroni corrected
^{x} - trend

Test variable	PC 1 $_{\text{main traits}}$			PC 2 $_{\text{main traits}}$		
Test run	n	t	p	n	t	p
1	15	-0.389	0.7031	14	1.5231	0.1517
2	13	-1.1056	0.2906	15	1.2036	0.2487
3	15	1.4902	0.1584	15	-1.3235	0.2069
4	14	0.3876	0.7046	13	0.0785	0.9387
5	15	2.4366	0.144$^{\#}$	15	1.8893	0.0797^{x}
6	14	3.166	0.0414$^{\#*}$	15	2.4664	0.1632$^{\#}$

Table 15 Detailed statistics on assortative mating analysis PC1$_{\text{main traits}}$ and PC2$_{\text{main traits}}$.
$^{\#}$- Holm-Bonferroni corrected
*- significant, ^{x} - trend

V. DEMOGRAPHIC AND INFECTION PATTERNS IN A LUMBRICID-GREGARINE SYSTEM UNDER ORGANIC AND NO TILLAGE MANAGEMENT

ABSTRACT

Earthworms are key organisms in agro-ecosystems. Their activity largely determines the soil´s conductive capacity, thus preventing erosion. The earthworm *L. terrestris* is a deep-burrowing species with vertical burrows, and is common in clay soil. I compared individuals from no tillage and organic fields in terms of several phenotypic parameters including infection with *Monocystis* (Gregarinidae, Apicomplexa). In addition, I used microsatellite markers for *L. terrestris* and a mitochondrial marker for *Monocystis* to infer genetic population composition and diversity. Demography differed between field types such that worms in organic fields are older and more often show shortened bodies, a consequence of ploughing-caused tail-loss. Infection patterns did not differ between field types, but I could show an association between one microsatellite allele and *Monocystis* strain abundance. I discuss the relevance of my findings for agricultural practitioners who attempt to undergo a change towards no tillage management.

INTRODUCTION

Earthworms are vital for soil life

It is now widely acknowledged that earthworms are vital for life in arable soil and that they positively influence soil fertility and plant growth (Scheu 2003). They dominate the biomass in soil fauna (500-1000 kg fresh weight / ha, (Lee 1985), have a high consumption rate and the production of biogenic structures such as burrows and casts provide new habitats for soil flora and fauna (Lee 1983; Anderson 1988; Lavelle, Bignell et al. 1997). Their activity promotes plant growth via various mechanisms: Plants may benefit from indirect effects such as hormone-like substances secreted by the earthworm´s epidermis and the dispersal of beneficial microorganisms (Brown 1995; Scheu, Schlitt et al. 2002; Scheu and Setala 2002). But more important are direct physical mediations between soil and plant life. Earthworms are primary decomposer and play a major role in the disintegration of plant residuals (Scheu and Setala 2002), thereby increasing the mineralization of N and P. These elements can then be taken up by plants, which will result in greater biomass production in the presences of earthworms (Devliegher and Verstraete 1997; Brussaard 1999; Scheu 2003; Eriksen-Hamel and Whalen 2007). Moreover, their activity alters soil properties such as soil aggregation, hydrodynamic properties (Shipitalo and Butt 1999) and aeration of deeper soil layers (Shipitalo and Le Bayon 2004), and they are therefore called “ecosystem engineers” (Lavelle, Bignell et al. 1997).

Earthworms are sensitive to human influences

The general constitution of an ecosystem is often indicated by the earthworm number and community living therein (Lee 1985; Hendrix 1998; Paoletti 1999). In supportive environments, populations can comprise several hundred individuals per square meter, while earthworms can be completely missing if conditions are unfavourable (Lee 1985). Whether or not and how chemical pesticides affect earthworms is controversial: On the one hand, they are directly exposed to the surrounding soil and could react to changing soil pH and Ca^{2+}. On the other hand, food availability seems to be one of the most limiting factors for earthworm populations. Their primary nutrition source, plant residue and soil organic matter, is reduced by the use of

herbicide and chemical fertilizer (Lee 1985; Pfiffner 1993; Estevez, Ndayegamiye et al. 1996; Paoletti 1999).

Recent empirical evidence however proposes that the biggest human impact on earthworm populations does not stem from lack of nutrition, but is due to physical damage of earthworm bodies and their habitat during soil cultivation (Eriksen-Hamel, Speratti et al. 2009; Ernst and Emmerling 2009). Tillage directly kills earthworms by chopping and immediate mortality is estimated between 25% (Curry, Byrne et al. 2002) and 70% (Bostrom 1988) per tillage run. Additionally, worms are exposed to predators (Cuendet 1983) and desiccation (Wyss and Glasstetter 1992; Edwards, Bohlen et al. 1995; Holland 2004; Jordan, Miles et al. 2004; Ernst and Emmerling 2009). In some cases, tillage may reduce populations by 50% or more compared to no-till systems (Mele and Carter 1999; Johnson-Maynard, Umiker et al. 2007). Intensive tillage can eliminate earthworm populations within a single season (Curry, Byrne et al. 2002), also by severely destroying their habitat. In top soil layers, tillage diminishes soil moisture, and a lack of cover residuals facilitates evaporation even more (Edwards, Bohlen et al. 1995; Clapperton, Miller et al. 1997; Curry 1998). Earthworms inhabiting deeper soil layers, and especially those who depend on permanent burrows, suffer from the destruction of their tunnels (Jordan, Miles et al. 2004; Capowiez, Cadoux et al. 2009) through ploughing.

Management types affect soil structure and earthworms

The use of the plough has been connected with soil cultivation ever since mankind adopted agriculture (Lal, Reicosky et al. 2007). In the last decades, extensive agro-industry was developed to meet the nutritional and economic needs of a growing world population. Application of herbicides and artificial fertilizer as well as tillage has severely deteriorated soil and water quality and earthworm populations alike. Environmental and health problems raised the consumer´s demand for sustainable food sources and lead to the adoption of organic farming. This management type relies on natural pest controls and organic fertilizers (Stolze, Piorr et al. 2000; Bond and Grundy 2001; Hansen, Alroe et al. 2001) like manure, dung and slurry, and this organic carbon input seems to be the reason why earthworms find better conditions in organic fields: No addition of this nutrition source and banning pesticides alone does not result in higher earthworm abundance (reviewed in Hole, Perkins et al. 2005; Pelosi, Bertrand et al. 2009). Nevertheless, earthworm density is in general

considered 2-3 times higher in organic than in conventional fields (Hansen, Alroe et al. 2001).

In organic farming, “natural” weed control often equals “physical” weed control: Ploughing is and has been traditionally the most effective way to burry weeds and to maintain an even seedbed (Triplett Jr and Dick 2008). Machinery that is commonly used for ploughing derives from conventional tillage and Koepke (2003) describes that organic farmers even till to deeper soil layers than conventional farmers. For more effective ploughing, new powerful engines were developed (Basch, Geraghty et al. 2008), but constant tillage by the mouldboard plough has severe consequences for the soil structure (Strudley, Green et al. 2008): Plough pans form a compacted layer beneath the topsoil and are impenetrable for water (Basch, Geraghty et al. 2008). The loosened topsoil with decreased pore volume and limited water retention capacity is then prone to erosion particularly in heavy rainfall (Triplett Jr and Dick 2008).

Soil degradation due to intensive tillage and erosion could cost the EU up to 38 billion € annually (EU commission report 2006), the main reasons why conservation tillage systems such as reduced or no tillage have come into focus in recent years (Holland 2004). In no tillage (also called direct sowing) management, the seeds are sown in with a direct sowing machine that slits the soil open and deposits the seed underground (invented by John Deere 1953). After harvest, residuals are chopped and spread over the field as cover. However, application of a mild herbicide is necessary to suppress weed growth. The practice has several advantages for both soil biota and cultivator: Less manpower, fuel and machinery are necessary and the soil structure is left intact such that it is evenly compacted (Triplett Jr and Dick 2008). A firm soil texture not only aids agricultural machine traffic, but also activates nutrient cycling and the formation of stable aggregates through stimulation of the soil food web (Franzluebbers 2002; Holland 2004; Bünemann, Schwenke et al. 2006).

Active soil life is fostered by no tillage management through chopped crop residues (Triplett Jr and Dick 2008). Primarily left on the surface as bedding to keep the top layer moist and prevent runoff and weed growth (Franzluebbers 2002), organic mulch serves as an excellent food supply for earthworms (Jordan, Miles et al. 2004; Coq, Barthès et al. 2007; Eisenhauer, Marhan et al. 2008; Eriksen-Hamel, Speratti et al. 2009). Their numbers therefore drastically increase under no tillage management

(Langmaack 1999; Shuster and Edwards 2003), and this effect can be mainly accredited to proliferation of anecic and endogeic species (Pelosi, Bertrand et al. 2009)

Earthworms as key organisms in NT farming

Those earthworm populations are not only favoured by consequent no tillage farming, but compliance of soil protection challenges also strongly depends on their contribution: Presence and species composition of earthworms can influence gas flux, soil structure and water drainage (Protz, Fox et al. 2000; Rizhiya, Bertora et al. 2007; Capowiez, Cadoux et al. 2009). Water infiltration rates of no tillage agro-ecosystems is six times higher (Ehlers 1975) than in conventional fields due to the burrow systems of anecic species (Lachnicht, Parmelee et al. 1997; Shipitalo and Le Bayon 2004; Lindahl, Dubus et al. 2009), who feed and mate on the surface but live below-ground in primarily vertical burrows (Edwards, Bohlen et al. 1995). Burrows can reach deep into the subsoil and are stabilized by microbial polysaccharides that form aggregates of insoluble organic matter lining burrow walls (Zhang and Schrader 1993; Oyedele, Schjønning et al. 2006; Bottinelli, Hallaire et al. 2010). These continuous macropores (Capowiez, Cadoux et al. 2009; Kay and Munkholm 2011) bypass the soil unsaturated zone and guide incoming rainwater directly to groundwater reservoirs (Lachnicht, Parmelee et al. 1997; Strudley, Green et al. 2008) thus preventing runoff and erosion.

Leaving soil-protecting mulch on the surface has been criticized because it facilitates the spread of the “head blight disease”, a fungal infection of grains and leafs by *Fusarium* species in Germany, France and Switzerland (Basch, Geraghty et al. 2008). *Fusarium* sp. feeds on dead plant material during non-vegetative periods after autumn harvest, ready to infest the new crop from spring onwards when residuals are not ploughed into the soil (Oldenburg, Kramer et al. 2008). Earthworm activity phases are highest during mild, moist weather in spring and autumn and they quickly burry crop residuals underground (Gallagher and Wollenhaupt 1997). Oldenburg, Kramer et al. (2008) could even show that earthworm preferentially ingest *Fusarium* infected straw, thus helping to prevent the spread of fungal spores (Oldenburg, Kramer et al. 2008). *Fusarium* produces a mycotoxin (DON) detrimental to cattle and humans in high doses. Therefore, official concentration thresholds regulate how much DON is allowed in crops designated to consumption (www.Eurowheat.com), and farmers

have to accept lower prices for their infected harvest. This is a major economic problem during transition from tillage to no tillage management because the biological system needs several years to find a new balance (Tebrügge and Düring 1999).

Estimates for the critical transition period range from 2 years (Pizl 1992) to 5 years (S. Weller, personal communication with NT farmer), depending on soil and weather conditions. A main factor that determines transition time to no tillage is how and which earthworm species recover. In a field experiment, (Johnson-Maynard, Umiker et al. 2007) was able to show that the earthworm population under no tillage management increased significantly over conventional tillage after 3 years. In the same order of magnitude are the findings of (Schmidt, Nitzsche et al. 2003) with an increase of earthworm densities from 211 to 572 individuals / m^2 after transition to no tillage winter wheat. It is, however, not completely clear how earthworms re-colonize fields, but it is likely that higher reproduction and uninterrupted dispersal can follow after reduction of disturbance. Which of the two mechanisms is more influential highly depends on the characteristics of the species (Decaëns and Jiménez 2002). Generally, for successful transition, an increase in anecic species is desirable and has been documented (Pfiffner and Luka 2007; Peigné, Cannavaciuolo et al. 2009).

L. terrestris reproduction limits transition to NT

The anecic earthworm *L. terrestris L*. (Clitellata, Oligochaeta) is a keystone species in no tillage management (Lee 1985). It lives in permanent vertical burrows that usually have only one opening to the surface, making it virtually sessile when it comes to spatial distribution (Nuutinen and Butt 2005). Nocturnal activities take mostly place on the surface and include foraging for litter, mate search and mating. During copulation, earthworms stay anchored in their burrows with their caudal ends while the front ends are attached in a typically S-shaped position. Retraction into the burrow is much slower in mating pairs than in single individuals and the pair has to detach by force, sometimes resulting in one, usually the smaller, of them becoming pulled out of its burrow. As copulations usually start between 2 and 4 o´clock in the morning and last about 2-6 hours, mating pairs and individuals stranded on the surface are conspicuous for predators, e.g. birds (Michiels, Hohner et al. 2001). Therefore, mating over long distances, which is likely at low densities, is risky and usually avoided (Sahm 2009 and Chapter IV). Transferred allosperm can be used for

fertilization for several months. Reproduction is generally very slow compared to other earthworms: They produce on average only one cocoon every week (in the active season), out of which a single young (twin rate 1%) hatches. With a generation time of 6 months (under optimal laboratory conditions) and the potential to live for several years (Butt 1993), *L. terrestris* is highly adapted to undisturbed, mild habitats. After years of tillage, population recovery and resulting benefits for soil protection may therefore require a farmer´s patience.

L. terrestris is parasitized by the gregarine apicomplexan *Monocystis sp*., which lives mainly in the seminal vesicles where self-sperm is stored. It is able to castrate the worm when parasite cells occur in high densities and move to the testes (Sims and Gerard 1985; Breidenbach 2002), but empirical studies on its fitness effects are contradictory: In two recent studies, I detected a direct effect of *Monocystis* concentrations on sperm cell abundance in the seminal vesicle (Chapter III and IV). Furthermore, some authors found a negative effect on growth (Field and Michiels 2005), while others didn´t (Field, Schirp et al. 2003). It is generally acknowledged that the *Monocystis-Lumbricus* system has coevolved for a long time, resulting in reduced parasite virulence and according host tolerance (Bush 2001). Nevertheless, *L. terrestris* shows considerable immune responses against *Monocystis* sporocysts, which may imply energetic costs of infection (Rolff and Siva-Jothy 2003; Field, Kurtz et al. 2004) The underlying genetics of both parasite virulence and host immunity are not yet completely understood, but continuous host-parasite co-evolution along with low host dispersal rates could have led to local adaptation. Field, Lange et al. (2007) could not detect a relationship between genetic diversity and parasite load in different patches of an urban metapopulation. On an even smaller scale, such host-parasite genotype clusters were not detected in a German meadow, but substantial host differentiation is frequent on larger scales i.e. between earthworms of German and Canadian origin (Velavan, Weller et al. 2009).

Genetic analysis of *Monocystis* populations within a single host earthworm revealed that infection with multiple strains is common (Velavan, Schulenburg et al. 2010). It is worth mentioning that *Monocystis* is not transmitted during copulation, but via oral ingestion of cysts (Field and Michiels 2006).

Expectations and objectives

Frequent distribution of infective cysts through tillage may lead to different infection patterns under organic and no tillage management. Most studies concerning influences of management focus on earthworm abundance or species community. Few, if any, explore health or genetic composition of the species found in different agro-environments, although this might be crucial for understanding how earthworm populations deal with habitat disturbance. The aim of my study was to compare condition parameters of *L. terrestris* populations under organic and no tillage management. Furthermore, I investigated the underlying genetic structure of both earthworms and their *Monocystis* parasites. If no tillage fields were recently populated, one could expect to see bottleneck or founder effects.

Study design

I sampled earthworms from organic and no tillage fields in Wendelsheim, South-Western Germany. Loamy soil and increasing weather extremes have led to severe erosion although the soil type is preferred by *L. terrestris*. NT farming as a resolution has formerly been unprofitable due to small acre size resulting from centuries of gavelkind (equal division of land among all qualified heirs). A land consolidation and farmer´s associations has made the practice of NT farming profitable and it is now used for about 5 years. Alongside, organic farming has been practiced in the area for around 15 years.

Genetic structuring between earthworm populations from both field types was inferred using a modified version of the genetic markers developed by (Velavan, Schulenburg et al. 2007; Velavan, Schulenburg et al. 2010) for both host and parasite.

MATERIALS AND METHODS

Sampling Sites

The selection of fields was made in consultation with the official agricultural advisory service. The sampling sites are located near the villages Wendelsheim and Wurmlingen, Baden-Württemberg, Germany (48° 30´ N, 8° 56´E). In this region, the former custom that a family's real estate was equally divided among the heirs resulted in an increase of smaller and smallest lots over generations. Even after land consolidation, the fields remain rather small (1-5 ha). As large field areas are required to make the more expensive direct sowing machines effective, the no tillage method is rather uncommon in southern Germany. Nevertheless, one farmer has been using this method for about 10 years. His fields partially intermix with fields of an organic farmer (**Figure 1**).

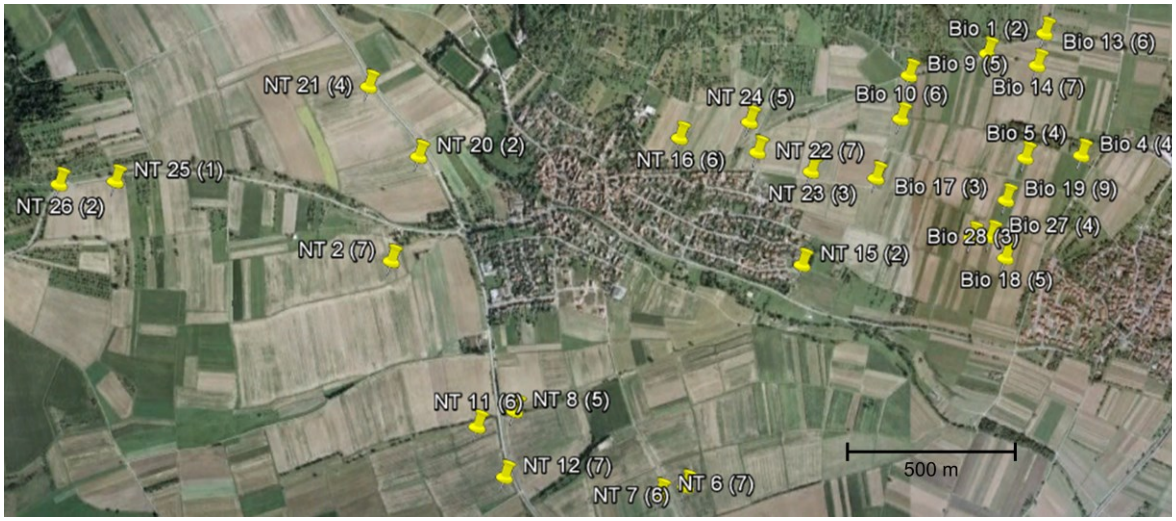

Figure 1 Map of sampling area, Bio=Bio, NT= No tillage, first number indicates field ID, number in brackets indicates no. of earthworms sampled.

Originally, I intended to include a third type of management, conventional farming. Plots that were conventionally farmed can be found intermixed with no tillage farming and organic farming. Unfortunately, I was not able to find any earthworms on these fields and therefore abandoned this third group. I decided to only sample on fields sown with winter wheat. All fields have the same basic soil conditions: a very thick and loamy soil, which is usually a preferred habitat for *L. terrestris*. I found *L. terrestris* in high densities on lawns near the fields.

Sampling Methods

Earthworms were sampled within a period of 4 weeks. In the first 2 weeks, I sampled earthworms via mustard expulsion. After heavy rain falls, this method was no longer suitable. I then collected earthworms by catching them at night, using special red light head torches as light source. Fields were sampled in a random order. I sampled 5-10 specimen per field with a minimum distance between burrows of 2 m. Worms were checked for maturity and placed into labelled vials. Their burrow entrance was marked with a wooden stick and a glow stick at night. I measured the distances between worms with a tape measure. GPS measurements were taken in the approximate centre of the sampled worms. Worms were stored in the fridge at 8°C until the next morning. They were then washed, weighed and and cut behind the clitellum. Both parts were stored in 70% ethanol. All earthworms received a random number for further processing and were placed in labelled 35 ml Falcon tubes in 70% ethanol and stored at -20°C.

Processing Earthworms

Earthworms were dissected and sperm and parasites counted as described in Chapter III. Briefly, the seminal vesicles were removed, weighed, homogenized and diluted. Subsequently, sperm and parasite concentration were determined using a Thoma counting chamber. Furthermore, I counted all segments of the anterior part behind the clitellum under a stereo-microscope.

Genotyping of Lumbricus terrestris and Monocystis

Tissue DNA was extracted using the DNeasy Blood and Tissue kit (Quiagen). 25 mg of tail tip tissue were used for DNA extraction. The last step of the protocol was modified: Only 30 µl of Elution buffer were used for the elution. Three microsatellite loci developed by (Velavan, Schulenburg et al. 2007) were used: LTM163, LTM128 and LTM208. In order to make amplification and scoring more efficient, several optimizations were necessary: For LTM163, the annealing temperature was reduced to 58.7°C, final elongation was 10 min. For LTM128, the PIG-tailing of the forward primer was removed. Genomic DNA was diluted 1:10. A touchdown PCR from 60-55°C was used for annealing and final elongation was 10 min. PCR products were diluted 1:30 in ddH2O and analysed on an ABI 3031xl (Applied Biosystems) along

with a size standard (Rox 500). Fragment length was scored using the program Genmapper ® 3.1 (Applied Biosystems).

Monocystis DNA extraction was achieved using the protocol of (Holm 1979). After bleaching away host tissue, the remaining sporocysts were broken open with massive ultra-sonication. A modified version of the DNeasy blood and tissue kit was then used to extract the *Monocystis* DNA. Velavan, Schulenburg et al. (2010) describe the internal transcribed spacer (ITS) region of the ribosomal cistron of *Monocystis* sp. MITS. They use a page gel approach to determine the length of this region as a basis to discriminate between different *Monocystis* strains. In order to simplify genotyping, we modified the protocol using a fluorescent primer and determine MITS length on a Sequencer.

For the PCR reaction, at the 5' end of the forward primer we added the M13 sequence. PCR was then performed with this new primer [PCR conditions: 12.5 µl H_2O, 2 µl Buffer, 1 µl $MgCl_2$ 50 mM, 1 µl M13-MITS-Forward 10 mM, 1 µl MITS-Reverse 10 mM, 0.2 µl Taq, 0.8 µl DNTps 20 mM, 1.5 µl DNA, initial denaturation 5 min 94°C, 1 min 94 °C, 1 min 60°C, 1:15 min 72°C for 40 cycles, 7 min 72°C final annealing]. To remove impurities which could occur due to the mix of DNA of several Monocystis strains, PCR products were cloned into a bacterial vector using the pCR®-TOPO® kit (Invitrogen). Before sequencing, the resulting DNA was digested with EcoRI (8 µl H_20, 1.5 µl Buffer, 0.5 µl EcoRI enzyme, 5 µl DNA at 37°C for 1h. DNA was sequenced on an ABI 3210 sequencer.

Results were blasted and showed a 100% match with the target fragment MITS. To label the fragment with a fluorescent primer, we added 6FAM-M13 primer and redefined PCR conditions with an excess of 6FAM-M13 primer (12.2 µl H_2O, 2 µl Buffer, 1 µl $MgCl_2$ 50 mM, 0.8 µl M13-MITS-Forward 1 mM, 0.8 µl MITS-Reverse 10 mM, 0.72 µl 6FAM-M13 10mM, 0.2 µl Taq, 0.8 µl dNTPs 20 mM, 1.5 µl DNA) In the first PCR steps, M13-MITS-Forward primer will bind to the template DNA, then 6FAM-M13 primer will replace and continue the PCR reaction instead of the M13-MITS-Forward primer. The length of the resulting PCR products were determined by running them on the ABI 3210 sequencer along with the GeneScan™ 1200 LIZ® Size Standard LIZ1200 (Applied Biosystems).

Statistical analysis

In total, I sampled 218 individuals, 96 individuals from 12 organic field and 122 individuals from 15 no tillage fields. 19 individuals were excluded as juveniles because their clitellum was not fully developed. In 31 individuals, one or more microsatellites could not be amplified by PCR, and in 41 individuals, DNA extraction or amplification of MITs markers for *Monocystis* failed, leaving 127 individuals with complete datasets for the following analyses, 57 individuals from 12 organic fields and 70 individuals 15 from no tillage fields. Phenotypic data were ln(x) transformed to obtain normally distributed data (labelled with $^{\#}$).

Population genetic analysis of the microsatellite data was achieved using the program GenoDive (Meirmans and Van Tienderen 2004). Pairwise F_{st} values measure the diversity of a randomly chosen allele within the population of a field relative to diversity in both fields. F_{st} values can take values from 0 (panmixis) to 1 (complete separation). F_{st}s between fields ranged from 0.15 to 0.38 with $\bar{x} = 0.034$, indicating that only little genetic differentiation exists between fields. High levels of heterozygosity ($H_o$total = 0.69, range 0.44 to 1.0) and low inbreeding (G_{is}total = 0.004, range -0.5 to 0.4) further support the picture of a large, panmictic population.

Figure 2 Genetic clustering of sampling sites, first number indicates genetic cluster.

A possible migration barrier is the main street and the village crossing the sampling area from North-West to South-East (**Figure 2**). But even when clustering all individuals into two populations, a North-East and a South-West population, I did not find substantial population differentiation between them (F_{st} = 0.014). I assigned field

populations to clusters according to their microsatellite composition using the k-Means clustering method (AMOVA, simulated annealing with 50.000 steps). Best clustering was achieved with 4 clusters (AMOVA, SSD = 41.75, df = 23, rho = -0.04). Genetic clustering does not match spatial clustering (**Figure 2**), especially regarding to the above mentioned migration barrier. I conclude that migration must be frequent, possibly aided by the farming machinery that is used on both sides of the village. For statistical analysis of phenotypic data I employed a linear mixed model with maximum likelihood and “Field ID” as random factor using the program SPSS® 20.0. For the distribution of *Monocystis* strains, I corrected for differences in number of *Monocystis* strains within individual hosts. For each host, present *Monocystis* strains received an individual value “1/number of strains”, which was then used to calculate the overall relative strain abundance.

RESULTS

Phenotypic differences between organic and NT worms

The distribution of number of segments differs between the two field types: In organic fields, segment number showed a bimodal distribution with 11 individuals (19%) below 120 segments. In NT earthworms, only 6 individuals (9%) have fewer than 120 segments (**Figure 3**).

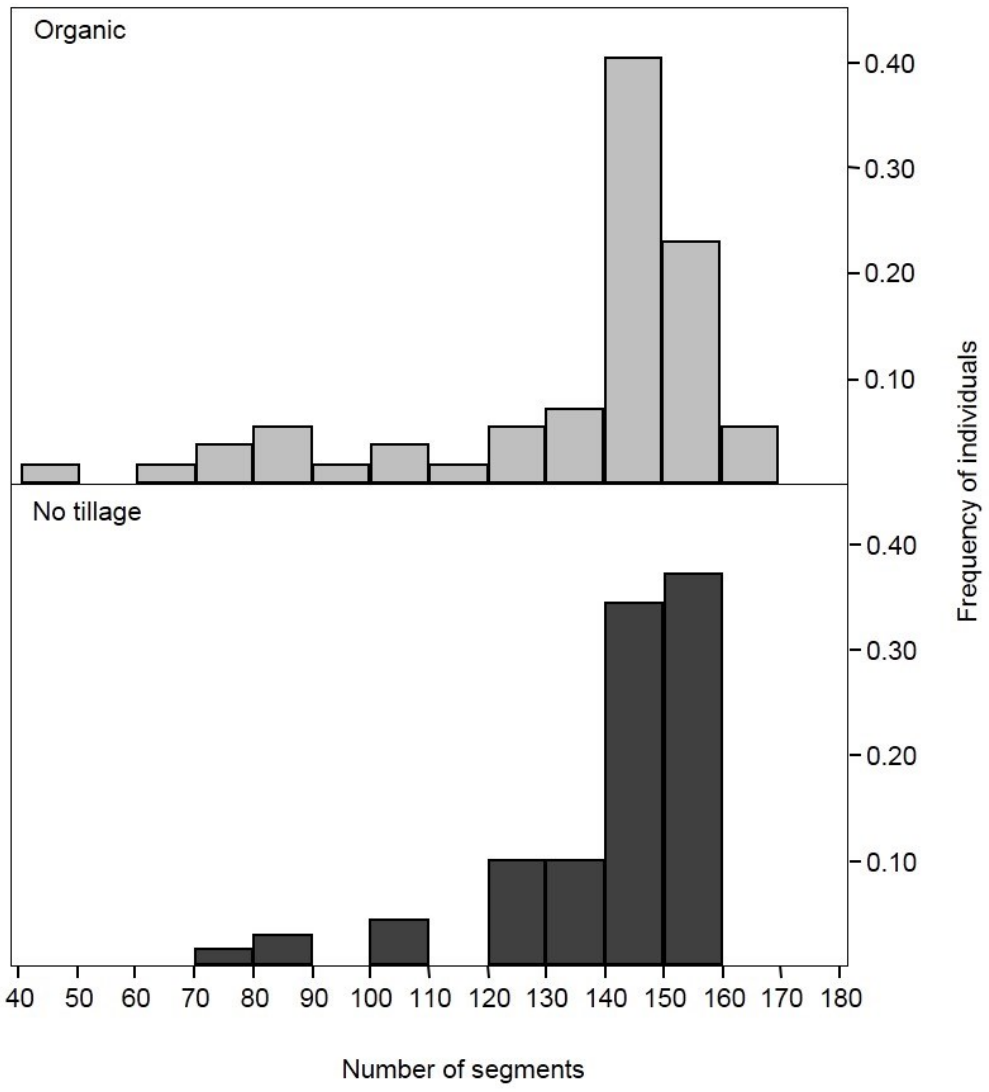

Figure 3 Distribution of number of segments in organic (top) and no tillage (bottom) fields.

The linear mixed model revealed substantial differences in vesicle weight between earthworms from organic and no tillage fields. The former have a higher vesicle weight (**Table 1**). Furthermore, I used the ratio "weight/segment" as an estimate for worm "thickness". Here, again, earthworms from organic fields have a higher weight/segment ratio, which means that they are generally thicker (**Figure 4**).

Variable [unit]	Effect of management type			Intercept			Estimated mean Bio	Estimated mean NT
	F	df	p	F	p	df		
Fresh weight [g]	6.0	22	0.11[+]	1094.3	>0.001	22	6.05	5.21
Vesicle weight [mg]	4.4	19	0.048[+*]	1093.6	>0.001	19	75.94	66.84
Total no. of parasites[#]	0.25	24	0.625	13985.5	>0.001	24	2.08×10^6	2.4×10^6
Total no. of sperm[#]	0.0	125	0.996	33268.2	>0.001	125	32×10^6	32×10^6
Weight / segment [g]	8.22	22	0.05[+*]	867.5	>0.001	22	0.046	0.038
No. of *Monocystis* strains	0.44	18	0.51	360.0	>0.001	18	6.60	7.08

Table 1 Results of the linear mixed model, $n_{bio} = 57$, $n_{nt} = 70$.
[#] data were ln(x) transformed for analysis
[+] p-values were Holm-Bonferroni corrected

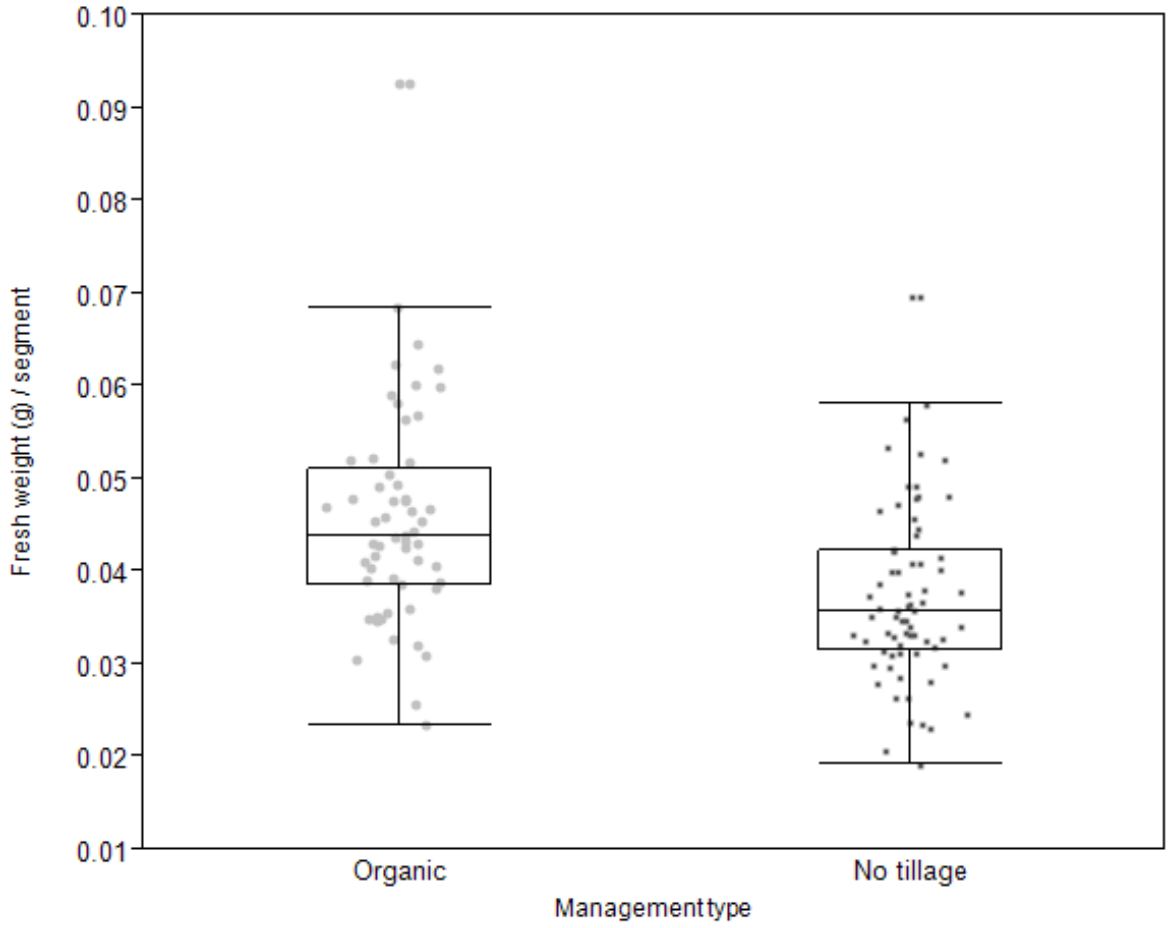

Figure 4 Difference in weight/segment (g) ratio between organic and no tillage earthworms. $n_{organic} = 57$, $n_{nt} = 70$.

Genetic structure of organic and no tillage populations

Organic and no tillage populations do not differ much in their genetic composition. Both show high genotype richness: Only few genotypes are present more than once, with the highest genotype abundance of only four. The three microsatellite loci I used are not equally diverse, with Ltm128 being the one with highest variation. But even Ltm208 shows enough variation to allow for population genetic analysis. Heterozygosity is high in both field types, and observed and expected heterozygosity do not differ from another, indicating that the population is in Hardy-Weinberg equilibrium. Inbreeding is extremely low in both field types, and a Shannon index that equals almost the natural logarithm of the Genotype Richness R also indicates that both populations are highly diverse (**Table 2**).

	Bio	No tillage
Genotype richness (R)		
No. of genotypes total [no. of worms]	42 [47]	46 [53]
ln (R)	3.73	3.83
No. of genotypes ltm 128 [no. of worms]	19 [57]	19 [68]
No. of genotypes Ltm 163	13 [56]	12 [68]
No. of genotypes Ltm 208	6 [48]	8 [57]
Heterozygosity		
Observed	0.722	0.681
Expected	0.722	0.732
Inbreeding (G_{is})	0.000	0.069
Shannon index	3.61	3.76

Table 2 Summary of population genetic parameters for bio and no tillage populations.

Allele frequencies of the three microsatellite loci were highly similar in both populations, except for allele 146 of locus Ltm163, which is more abundant in the no tillage population. It is worth mentioning that some rare alleles occur in only one management type: allele 169 of locus Ltm128, and alleles 192, 223, 263, 383 of locus Ltm208 occur only in the no tillage population and allele 236 of locus Ltm208 occurs only in organic populations (**Figure 5**).

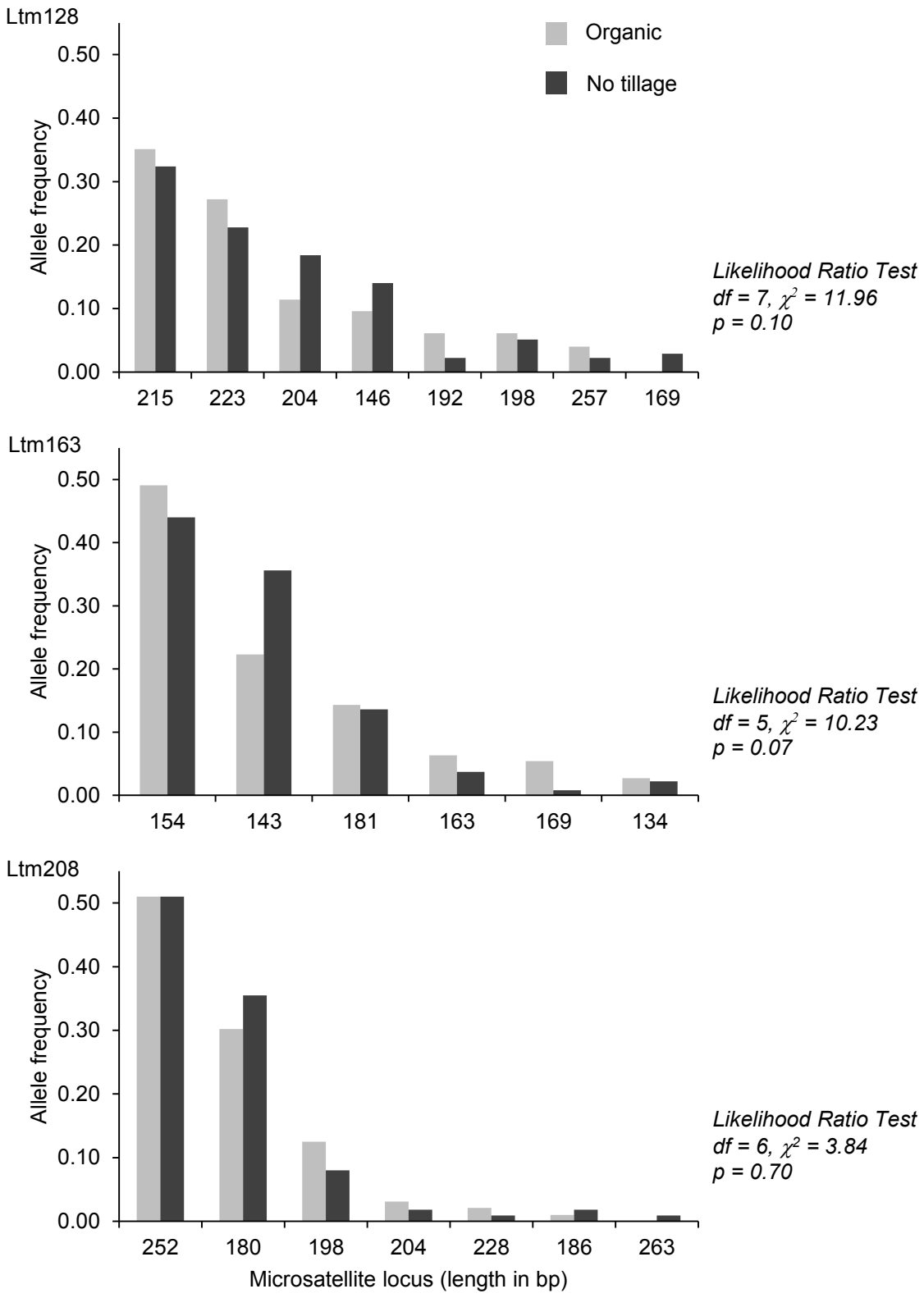

Figure 5 Allele frequencies for organic and no tillage populations for the 3 microsatellite loci.

Monocystis genetic structure in organic and no tillage populations

The parasite *Monocystis* turned out to be highly diverse in both host populations, with infection patterns ranging from 1 up to 15 parasite genotypes ($\bar{x}$ = 6.8, SD = 4.0). Organic and no tillage earthworms do not differ in the number of parasite strains they host (**Table 1**). In total, I found 32 different *Monocystis* strains, with some rare of them only present in the no tillage (660, 722, 756, 780, 908, 927 and 985) and one only present in the organic population (972). There is, however, no substantial difference in MITS allele frequencies between the two management types (**Figure 6**).

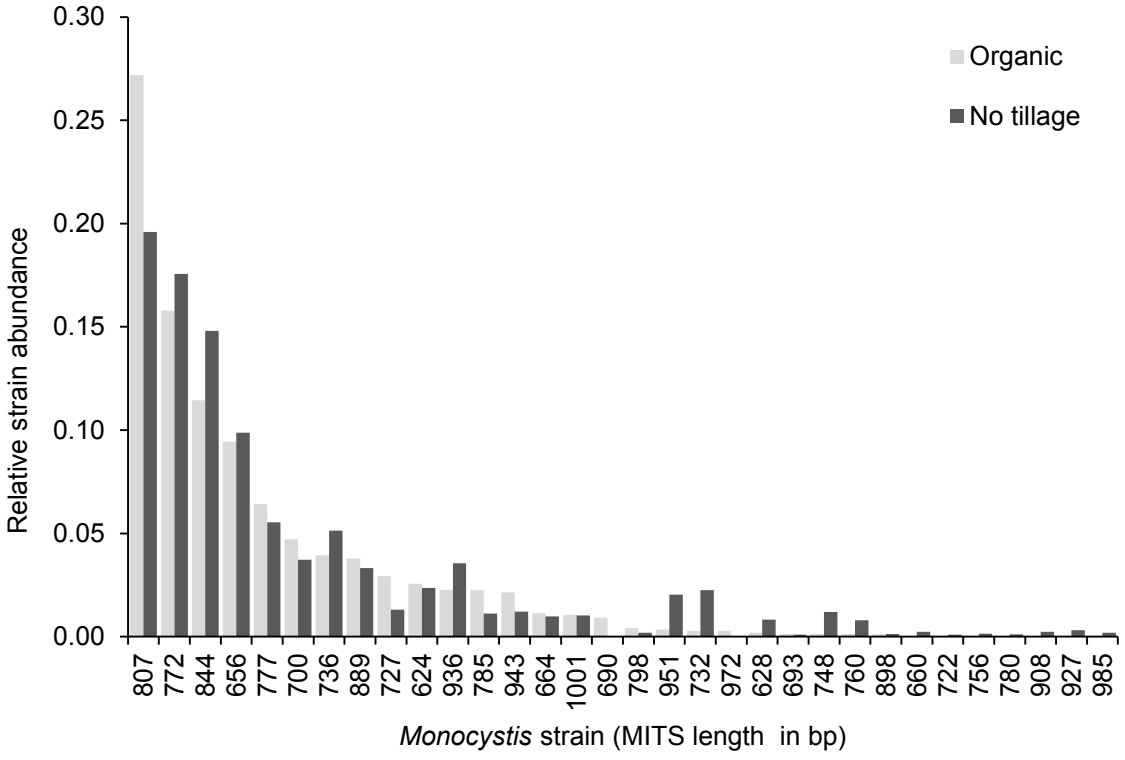

Figure 6 Relative strain abundance per Microsatellite strain for organic and no tillage populations. Distributions do not differ from each other (*Kolmogorov-Smirnov Test, df = 31, D= 0.19, p = 0.58).*

Interaction between Ltms and MITS in organic and NT populations

In NT worms, there is a trend that the distribution of *Monocystis* strains differs across the host alleles Ltm163_143 + Ltm163_152 on the one hand and the allele Ltm163_181 on the other. Allele 181 of locus Ltm163 shows an association with the most common *Monocystis* strain (**Figure 7**).

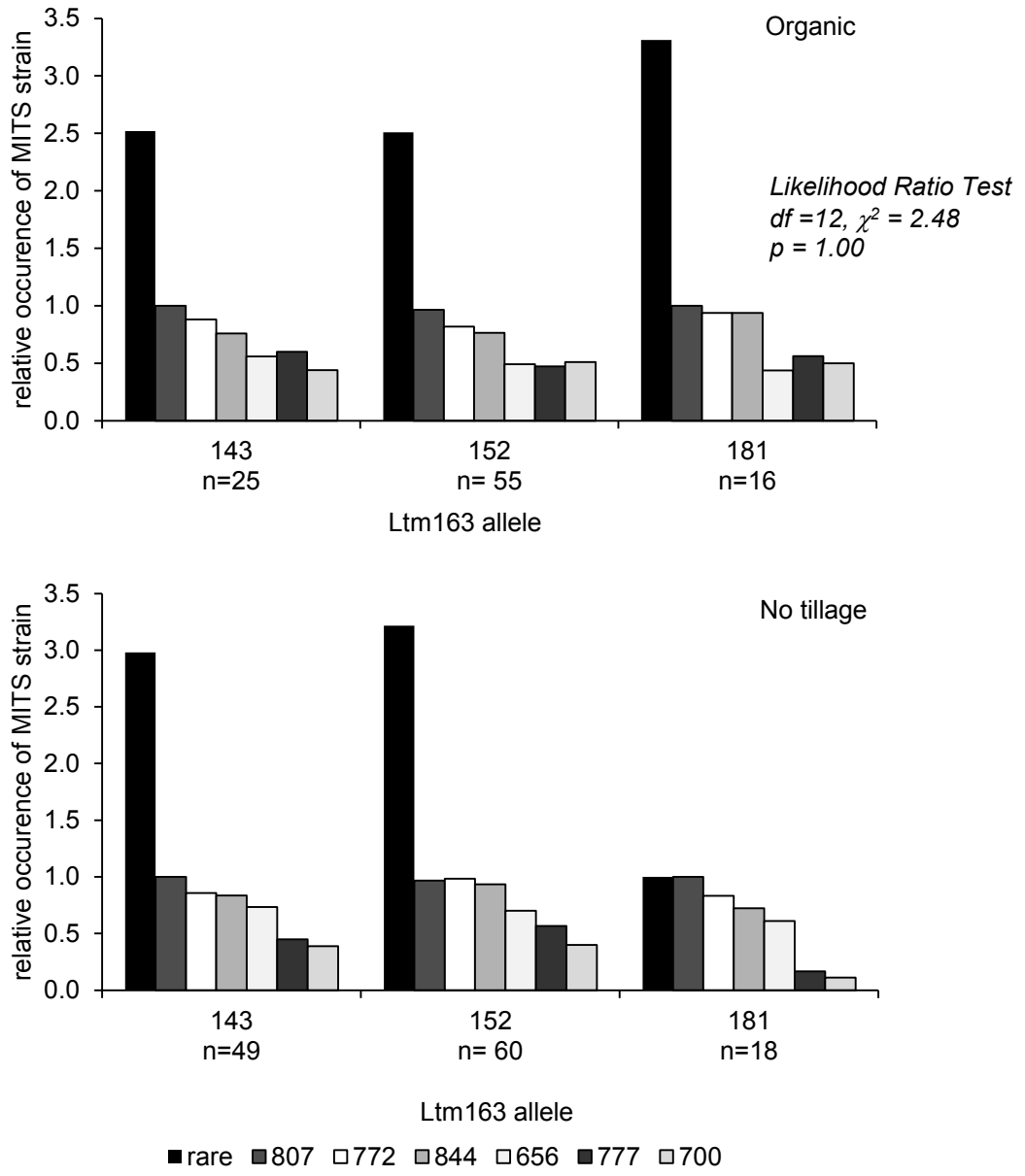

Figure 7 Relative occurrence of MITS strains per Ltm163 allele.

In no tillage fields, earthworms with the allele LTM163_181 harbour fewer *Monocystis* strains (**Figure 8**). Additionally, there is a trend that these individuals have a higher sperm concentration (**Figure 9**), although there is generally no correlation between no. of *Monocystis* strains and sperm or parasite concentration.

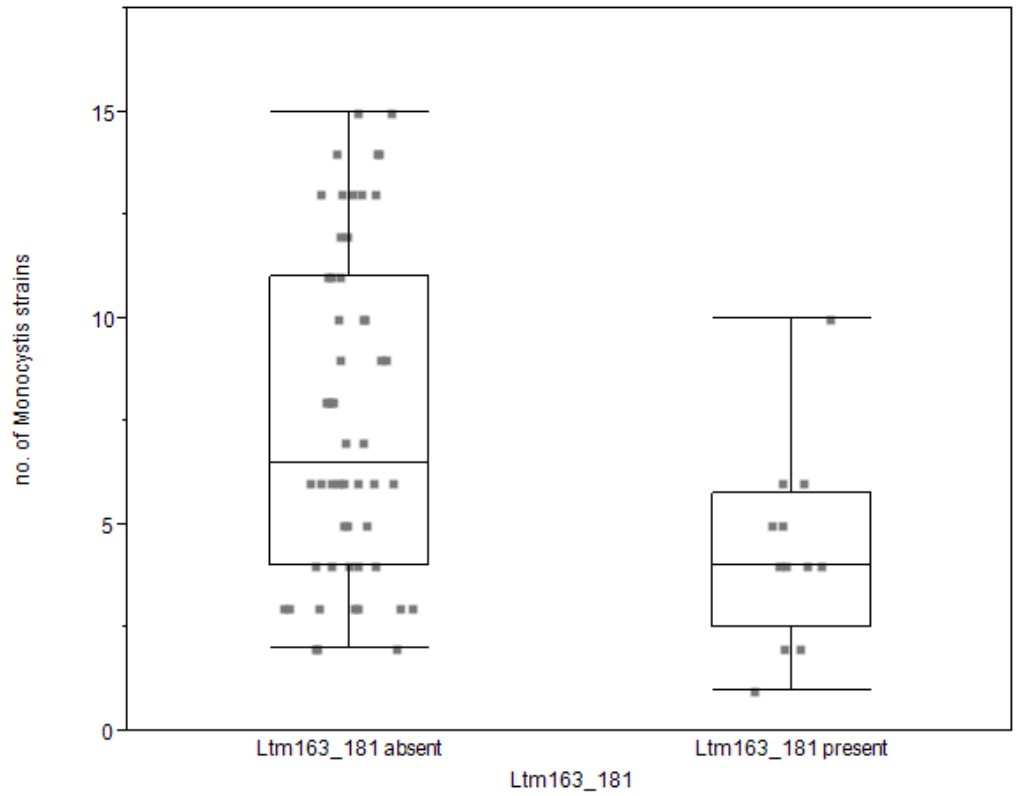

Figure 8 Difference in no. of *Monocystis* strains in earthworms without and with the allele Ltm163_181. (*Wilcoxon test,* n_{absent} *= 56,* $n_{present}$ *= 12, Z = 2.63, p = 0.0086*).

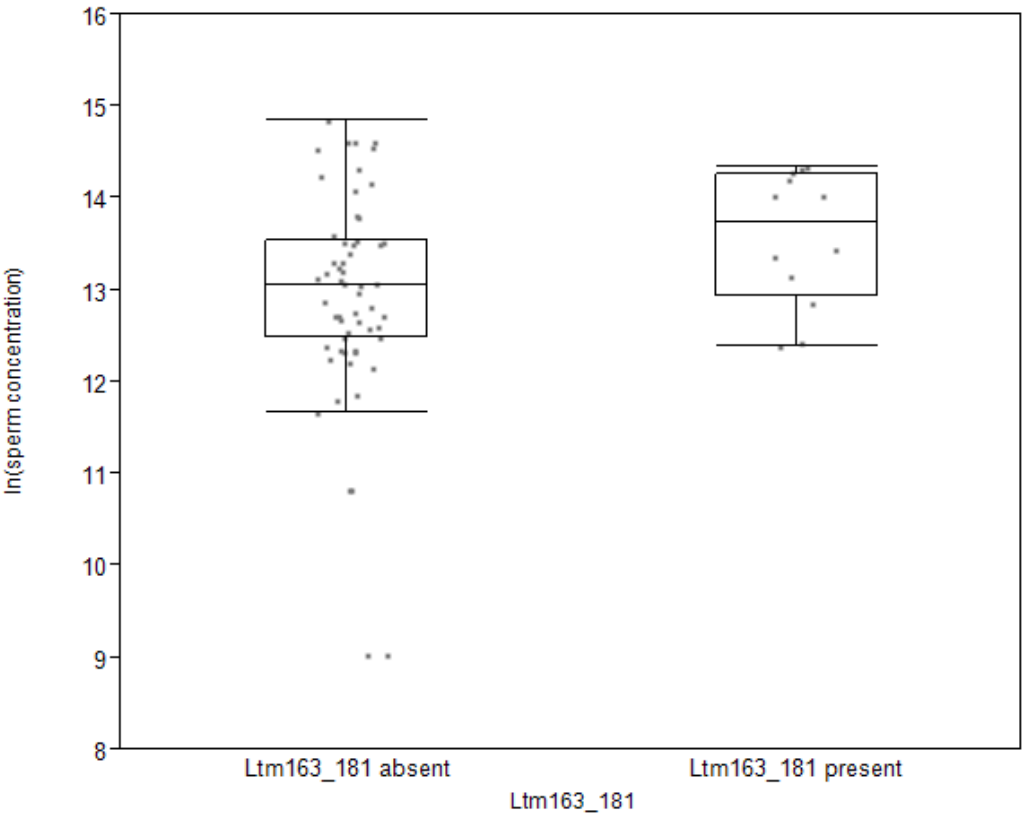

Figure 9 Difference in ln(sperm concentration) in earthworms without and with the allele Ltm163_181. (*Wilcoxon test,* n_{absent} *= 56,* $n_{present}$ *= 12, Z = 1.77, p = 0.076*).

DISCUSSION

Effects of management on physical appearance and demography

My results show that earthworm populations from organic and no tillage fields differ in their demographic structure. In organic fields, I found a cohort of short individuals with 40-100 segments, although organic worms have a higher weight/segment ratio and are thus thicker. I conclude that intensive tillage causes amputation of the posterior segments, which is also what I could observe during dissection and segment counting. No tillage earthworms have smaller weight/segment ratio, which means they are generally thinner. Additionally, they have smaller seminal vesicles (**Table 1**), an indication that they just recently matured. A large amount of semi-adults were found on no tillage fields and had to be excluded from the study. Conclusively, the no tillage populations seem to consist of young individuals, while organic populations are composed of old, thick individuals. These can survive tillage due to their deeper burrows (Pitkänen and Nuutinen 1997), but sometimes loose posterior segments either through (a) mechanical damage or (b) predatory birds that follow the traction engine.

High diversity in host and parasite in both field types

Genetic diversity is generally high in both field types. I could not observe a divergence from Hardy-Weinberg equilibrium in no tillage fields, which means that bottleneck or founder effects from repopulation events have already vanished or never occurred. Earthworms seem to have higher dispersal rates than expected (Velavan, Weller et al. 2009; Mathieu, Barot et al. 2010). Reports of triggered migration events e.g. due to low food or overcrowded habitats support this hypothesis (Mathieu, Barot et al. 2010, Grigoropoulou and Butt 2010). *L. terrestris* has long been seen as a semi-sessile species with individuals staying anchored in their burrow for feeding and copulation. In other experiments, I could observe that some individuals leave their burrow, copulate outside and then find back in again (Chapter III and IV). If they exert this behaviour in their natural environment, their mating range is much higher than previously assumed. Outbreeding is thus more likely and could help populations to maintain diversity even in founding situations with low density.

An alternative explanation for high diversity could be that the microsatellite markers that were used for this study have high mutation rates. This is rather unlikely because marker length seldomly differed by one, but usually by several repeats (**Figure 5**).

Infection patterns – a hint to an alternative infection mode?

Earthworms from organic and no tillage fields did not differ in the total amount of *Monocystis* spores they harboured (**Table 1**) although no tillage earthworms have smaller vesicles. I also found that *Monocystis* is highly diverse in both field types with 32 different strains detected. Although earthworms from no tillage fields are possibly younger, they might have been infected more rapidly. A dosage effect in the infection with *Monocystis* spores is likely and was encountered in pre-experiments for the artificial infection study (Chapter III), so how could no tillage earthworms ingest large amounts of spores? - The commonly described route of transmission for *Monocystis* spores is via ingestion of contaminated soil or faeces of vertebrates that fed on earthworms (Casemore 1991). In organic fields, a considerable proportion of worms is chopped up with every tillage run. This should lead to a higher prevalence of spores in the soil, and worms should easier get infected. In no tillage fields, earthworms stay intact until they die due to other causes. *Monocystis* could exert an alternative, more effective transmission mode: Juvenile earthworms can move into burrows that are no longer inhabited, but still contain the decayed remains of its former occupant. It is likely that the earthworm feeds on these remains, as earthworms particularly prefer to ingest smelly, protein-rich food (see Chapter III). The body of the dead earthworm presumably contains much higher concentrations of *Monocystis* spores than the mixed soil of organic fields, thus making infection more effective. The fact that no tillage worms live in high density, and that intact “Ghost burrows” have been reported in reduced tillage systems (Capowiez, Cadoux et al. 2009) supports this hypothesis.

Part of this result is due to the M13 method, which is generally more sensitive than the previously used page gel approach. The distribution of *Monocystis* strains (**Figure 6**) shows that there are 6 genotypes that are common and occur in almost every earthworm, while others are present only once. There are some rare genotypes that occur only in no tillage individuals, which is probably a sampling effect of higher sample sizes in no tillage fields. I could not detect a correlation between the number of *Monocystis* strains and infection load, but my approach does not allow for

quantitative analyses of *Monocystis* strains within individuals. Different amounts of the different *Monocystis* strains could have an effect on infection load or phenotypic measures. Cysts clearly differ in size (**Figure 7** in Chapter II), and this could be used for a quantitative discrimination, although it is still unclear if different sizes represent different strains.

Host allele effect on infection pattern in no tillage fields

I found that in no tillage fields, the allele Ltm163_181 is connected to a different *Monocystis* strain distribution than the other two alleles of the same locus. Individuals that have the Ltm163_181 harbour fewer rare *Monocystis* strains and at the same time have a trend towards higher sperm concentration. Although this effect was only present in 12 individuals, it is possible that multiple infections are less frequent in earthworms carrying the Ltm163_181 allele. To what extend the Ltm163 microsatellite marker is linked to resistance genes or parasite avoidance strategies still remains speculative and requires further investigation.

Which management type is better for earthworms?

The underlying question of this study was to see which management type promotes healthy earthworm populations. From a demographic perspective, I can conclude that organic fields are populated by old and damaged individuals, while no tillage populations consist of younger, often pre-mature earthworms. As I did not re-sample fields after a certain time period, I cannot infer how demographic patterns developed under the different management types. It seems, however, likely that earthworm populations in organic fields suffer from a lack of juveniles and do not have a possibility to replenish this loss. On the contrary, no tillage earthworms seem to meet favourable conditions for reproduction as is also indicated by their higher densities.

An issue that often arises from agricultural practitioners is where earthworms in no tillage fields come from. With my study, I cannot answer whether individuals immigrated or if populations recovered from within the fields. It shows, however, that, if there had been a bottleneck phase, a recovery period of 5 years seems to be enough to achieve high genetic diversity. These findings match with observations that converting to no tillage requires a period of 3-5 years to meet normal to high yields again, and would support the idea that the slow reproducing, deep burrowing species

are necessary for effective no tillage management and prevention of *Fusarium* growth.

Conclusion

In conclusion, tillage influences the demographic structure of earthworm populations. Generally, no tillage is beneficial for *L. terrestris* and favours population growth. It has been suggested that even on loamy and clay soil, reduced tillage can be an option for organic farming to preserve earthworm populations (Berner, Hildermann et al. 2008). *Monocystis* infection patterns are similar in both management types, which could be due to higher infection risks in no tillage populations. To what extend *Monocystis* strain infectivity and *L. terrestris* resistance interact needs further investigation.

APPENDIX

ID	1	2	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1		0.08	0.02	0.01	0.03	-0.05	0.04	0.15	0.08	0.14	0.03	0.06	-0.02	0.15	0.07	0.06	0.04	0.11	0.36	0.02	0.1	0.05	0.06	0	0.07	0.18	0.19
2	0.08		0.1	0.02	0.1	0.05	0.08	0.11	0.13	0.05	0.03	-0.02	0.06	0	0.02	0.02	0.06	0.01	0.21	-0.01	0.05	0	0.06	0	0.09	-0.02	-0.01
4	0.02	0.1		-0.06	0.06	-0.04	0.06	-0.08	-0.05	-0.01	-0.02	-0.03	-0.03	0.03	-0.02	0.05	-0.03	0.05	-0.06	0.04	-0.06	0.15	-0.05	0	0.03	0.08	0.06
5	0.01	0.03	-0.06		0.03	-0.04	0.05	-0.04	-0.02	0.05	-0.08	-0.06	-0.03	-0.07	-0.04	-0.03	-0.06	-0.01	-0.02	-0.04	-0.01	0.08	-0.09	0	0.01	0.04	0.07
6	0.03	0.01	0.06	0.03		-0.05	0.06	0.16	0.06	0.08	0.02	0.07	0.05	0.07	0.1	0.03	0.08	0.09	0.21	0.08	0.08	0.03	0.11	0	0.01	0.12	0.15
7	-0.05	0.05	-0.04	-0.04	-0.05		0.04	0.05	0.01	0.02	-0.04	0	0	-0.05	0.01	0.05	0.01	0.02	0.07	-0.02	0.04	0.05	0.02	0	-0.03	0.08	0.11
8	0.04	0.08	0.06	0.05	0.06	0.04		0.11	0.08	0.02	0.02	0.05	0	0.04	0.02	0.02	0.09	0.09	0.22	0.06	0.06	-0.15	0.07	0	-0.04	0.11	0.11
9	0.15	0.11	-0.08	-0.04	0.16	0.05	0.11		0.01	0.06	-0.01	-0.01	0.01	0.03	-0.02	0.11	-0.01	0.06	-0.09	0.02	0	0.21	-0.08	0	0.09	0.1	0.12
10	0.08	0.13	-0.05	-0.02	0.06	0.01	0.08	0.01		0.08	0	0.01	-0.01	0.06	0.02	0.04	-0.02	0.07	0.04	0.06	0	0.16	-0.04	0	0.08	0.1	0.16
11	0.14	0.05	-0.01	0.05	0.08	0.02	0.02	0.06	0.08		0.04	0	0.06	0.09	0.03	0.09	0.06	0.07	0.18	0.07	-0.01	0.08	0.07	0	0.05	0.04	0
12	0.03	0.03	-0.02	-0.08	0.02	-0.04	0.02	-0.01	0	0.04		-0.04	-0.01	-0.08	-0.02	0.01	-0.01	0	0.02	-0.04	0.01	0.03	-0.04	0	-0.03	0.03	0.08
13	0.06	-0.02	-0.03	-0.06	0.07	0	0.05	-0.01	0.01	0	-0.04		0.01	-0.07	-0.04	-0.03	-0.03	-0.03	0.05	-0.04	-0.03	0.03	-0.05	0	0.03	-0.06	-0.02
14	-0.02	0.06	-0.03	-0.03	0.05	0	0	0.01	-0.01	0.06	-0.01	0.01		-0.01	-0.02	0	0.01	0.02	0.07	0.02	0.01	0.02	-0.02	0	0.05	0.07	0.12
15	0.15	0	0.03	-0.07	0.07	-0.05	0.04	0.03	0.06	0.09	-0.08	-0.07	-0.01		-0.11	0.03	-0.05	-0.08	0.17	-0.12	0.07	0.01	-0.02	0	0.03	0.14	0.12
16	0.07	0.02	-0.02	-0.04	0.1	0.01	0.02	-0.02	0.02	0.03	-0.02	-0.04	-0.02	-0.11		0.02	-0.02	-0.04	0.05	-0.06	0.02	0.03	-0.04	0	0.01	0.06	0.09
17	0.06	0.02	0.05	-0.03	0.03	0.05	0.02	0.11	0.04	0.09	0.01	-0.03	0	0.03	0.02		-0.03	0.02	0.22	0.05	-0.02	-0.08	0.02	0	0.07	0.04	0.01
18	0.04	0.06	-0.03	-0.06	0.08	0.01	0.09	-0.01	-0.02	0.06	-0.01	-0.03	0.01	-0.05	-0.02	-0.03		0.02	0	-0.02	0	0.13	-0.05	0	0.04	0.05	0.07
19	0.11	0.01	0.05	-0.01	0.09	0.02	0.09	0.06	0.07	0.07	0	-0.03	0.02	-0.08	-0.04	0.02	0.02		0.13	-0.03	0.06	0.06	0.02	0	0.07	0.06	0.1
20	0.36	0.21	-0.06	-0.02	0.21	0.07	0.22	-0.09	0.04	0.18	0.02	0.05	0.07	0.17	0.05	0.22	0	0.13		0.08	0.08	0.38	-0.08	0	0.21	0.26	0.25
21	0.02	-0.01	0.04	-0.04	0.08	-0.02	0.06	0.02	0.06	0.07	-0.04	-0.04	0.02	-0.12	-0.06	0.05	-0.02	-0.03	0.08		0.07	0.04	-0.03	0	-0.01	0.08	0.1
22	0.1	0.05	-0.06	-0.01	0.08	0.04	0.06	0	0	-0.01	0.01	-0.03	0.01	0.07	0.02	-0.02	0	0.06	0.08	0.07		0.1	-0.01	0	0.08	-0.01	-0.03
23	0.05	0	0.15	0.08	0.03	0.05	-0.15	0.21	0.16	0.08	0.03	0.03	0.02	0.01	0.03	-0.08	0.13	0.06	0.38	0.04	0.1		0.1	0	-0.01	0.1	0.12
24	0.06	0.06	-0.05	-0.09	0.11	0.02	0.07	-0.08	-0.04	0.07	-0.04	-0.05	-0.02	-0.02	-0.04	0.02	-0.05	0.02	-0.08	-0.03	-0.01	0.1		0	0.04	0.03	0.1
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0
26	0.07	0.09	0.03	0.01	0.01	-0.03	-0.04	0.09	0.08	0.05	-0.03	0.03	0.05	0.03	0.01	0.07	0.04	0.07	0.21	-0.01	0.08	-0.01	0.04	0		0.18	0.13
27	0.18	-0.02	0.08	0.04	0.12	0.08	0.11	0.1	0.1	0.04	0.03	-0.06	0.07	0.14	0.06	0.04	0.05	0.06	0.26	0.08	-0.01	0.1	0.03	0	0.18		-0.04
28	0.19	-0.01	0.06	0.07	0.15	0.11	0.11	0.12	0.16	0	0.08	-0.02	0.12	0.12	0.09	0.01	0.07	0.1	0.25	0.1	-0.03	0.12	0.1	0	0.13	-0.04	

Table 3 Pairwise F_{st} values between all combinations of fields.

VI. LITERATURE

A

Amos, W., J. W. Wilmer, et al. (2001). "The influence of parental relatedness on reproductive success." Proceedings of the Royal Society of London. Series B: Biological Sciences 268(1480): 2021-2027.

Amotz, Z. (1975). "Mate selection—A selection for a handicap." Journal of Theoretical Biology 53(1): 205-214.

Anderson, J. M. (1988). "Invertebrate-mediated transport processes in soils." Agriculture Ecosystems & Environment 24(1-3): 5-19.

Andersson, M. B. (1994). Sexual selection, Princeton Univ Pr.

Andersson, M. B. and L. W. Simmons (2006). "Sexual selection and mate choice." Trends in Ecology & Evolution 21(6): 296-302.

Angeloni, L. (2003). "Sexual selection in a simultaneous hermaphrodite with hypodermic insemination: body size, allocation to sexual roles and paternity." Animal Behaviour 66(3): 417-426.

Angeloni, L., J. W. Bradbury, et al. (2003). "Multiple mating, paternity, and body size in a simultaneous hermaphrodite, Aplysia californica." Behav. Ecol. 14(4): 554-560.

Angeloni, L., J. W. Bradbury, et al. (2002). "Body size and sex allocation in simultaneously hermaphroditic animals." Behavioral Ecology 13(3): 419-426.

Anthes, N. (2010). Mate choice and reproductive conflict in simultaneous hermaphrodites

Animal Behaviour: Evolution and Mechanisms. P. Kappeler, Springer Berlin Heidelberg: 329-357.

Anthes, N., P. David, et al. (2010). "Bateman gradients in hermaphrodites: An extended approach to quantify sexual selection." The American Naturalist 176(3): 249-263.

Anthes, N. and N. K. Michiels (2007). "Precopulatory stabbing, hypodermic injections and unilateral copulations in a hermaphroditic sea slug." Biology Letters 3(2): 121-124.

Anthes, N., A. Putz, et al. (2005). "Gender trading in a hermaphrodite." Current Biology 15(19): R792-R793.

Anthes, N., A. Putz, et al. (2006a). "Sex role preferences, gender conflict and sperm trading in simultaneous hermaphrodites: a new framework." Animal Behaviour 72(1): 1-12.

Anthes, N., A. Putz, et al. (2006b). "Hermaphrodite sex role preferences: The role of partner body size, mating history and female fitness in the sea slug Chelidonura sandrana." Behavioral Ecology and Sociobiology 60(3): 359-367.

Anthes, N., H. Schulenburg, et al. (2008). "Evolutionary links between reproductive morphology, ecology and mating behavior in opisthobranch gastropods." Evolution 62(4): 900-916.

Arnold, S. J. (1994). "Bateman's principles and the measurement of sexual selection in plants and animals." The American Naturalist 144: 126-149.

Arnqvist, G. and T. Nilsson (2000). "The evolution of polyandry: Multiple mating and female fitness in insects." Animal Behaviour 60(2): 145-164.

Arnqvist, G., T. Nilsson, et al. (2005). "Mating rate and fitness in female bean weevils." Behavioral Ecology 16(1): 123-127.

Arnqvist, G. and L. Rowe (2005). Sexual conflict, Princeton University Press.

B

Barnes, R. S. K., C. P., et al., Eds. (1993). The invertebrates: a new synthesis. Journal of Evolutionary Biology, Blackwell Science Ltd.

Barry, K. L. and H. Kokko (2010). "Male mate choice: why sequential choice can make its evolution difficult." Animal Behaviour 80(1): 163-169.

Basch, G., J. Geraghty, et al. (2008). "No-tillage in Europe–state of the art: constraints and perspective." No-Till Farming Systems(3): 159-168.

Bateman, A. J. (1948). "Intra-sexual selection in Drosophila melanogaster." Heredity 2(2): 277-277.

Bateman, P. W. and P. A. Fleming (2006). "Males are selective too: Mating, but not courtship, with sequential females influences choosiness in male field crickets (Gryllus bimaculatus)." Behavioral Ecology and Sociobiology 59(4): 577-581.

Baur, B., R. Locher, et al. (1998). "Sperm allocation in the simultaneously hermaphroditic land snail Arianta arbustorum." Animal Behaviour 56(4): 839-845.

Bedhomme, S., G. Bernasconi, et al. (2009). "How does breeding system variation modulate sexual antagonism?" Biology Letters 5(5): 717-720.

Bedhomme, S., G. Bernasconi, et al. (2009). "How does breeding system variation modulate sexual antagonism?" Biology Letters 5(5): 717-720.

Beese, K., K. Beier, et al. (2006). "Coevolution of male and female reproductive traits in a simultaneously hermaphroditic land snail." Journal of Evolutionary Biology 19(2): 410-418.

Bell (1982). "The masterpiece of nature: The evolution and genetics of sexuality." University of California press.

Berlin, H. (1923). Untersuchungen über Monocystidéen in den Vesiculae seminales der schwedischen Oligochaeten, C. Blom.

Berner, A., I. Hildermann, et al. (2008). "Crop yield and soil fertility response to reduced tillage under organic management." Soil and Tillage Research 101(1–2): 89-96.

Birkhead, T. R., F. Fletcher, et al. (1998). "Sexual selection in the zebra finch Taeniopygia guttata: Condition, sex traits and immune capacity." Behavioral Ecology and Sociobiology 44(3): 179-191.

Blows, M. W., S. F. Chenoweth, et al. (2004). "Orientation of the genetic variance-covariance matrix and the fitness surface for multiple male sexually selected traits." The American Naturalist 163(3): 329-340.

Bond, W. and A. C. Grundy (2001). "Non-chemical weed management in organic farming systems." Weed Research 41(5): 383-405.

Borgia, G. and K. Collis (1989). "Female choice for parasite-free male satin bowerbirds and the evolution of bright male plumage." Behavioral Ecology and Sociobiology 25(6): 445-453.

Bos, D. H., R. N. Williams, et al. (2009). "Condition-dependent mate choice and a reproductive disadvantage for MHC-divergent male tiger salamanders." Molecular Ecology 18(15): 3307-3315.

Bostrom, U. (1988). Population dynamics and activity in four cropping systems. Ecology of earthworms in arable land

Bottinelli, N., V. Hallaire, et al. (2010). "Abundance and stability of belowground earthworm casts influenced by tillage intensity and depth." Soil and Tillage Research 106(2): 263-267.

Bradbury, J. W., M. B. Anderson, et al. (1987). "Sexual selection: Testing the alternatives." Journal of Evolutionary Biology 2(1): 72-74.

Brauer, V. S., L. Schärer, et al. (2007). "Phenotypically flexible sex allocation in a simultaneous hermaphrodite." Evolution 61(1): 216-222.

Breidenbach, J. (2002). "Normalanatomie und- histologie des Lumbriciden Lumbricus terrestris L. (Annelida: Oligochaeta)." Doktoral thesis, University of Münster, Münster, Germany.

Bremner, A. A., E. A. Trippel, et al. (2002). "Sound production by adult haddock, Melanogrammus aeglefinus, in isolation, pairs and trios." Environmental Biology of Fishes 65(3): 359-362.

Brooks, R. and J. A. Endler (2001). "Direct and indirect sexual selection and quantitative genetics of male traits in guppies (Poecilia reticulata)." Evolution 55(5): 1002-1015.

Brown, G. G. (1995). "How do earthworms affect microfloral and faunal community diversity?" Plant and Soil 170(1): 209-231.

Brown, G. R., K. N. Laland, et al. (2009). "Bateman's principles and human sex roles." Trends in Ecology & Evolution 24(6): 297-304.

Brussaard, L. (1999). "On the mechanisms of interactions between earthworms and plants." Pedobiologia 43(6): 880-885.

Bulmer, M. G. and G. A. Parker (2002). "The evolution of anisogamy: A game-theoretic approach." Proceedings of the Royal Society of London. Series B: Biological Sciences 269(1507): 2381-2388.

Bünemann, E. K., G. D. Schwenke, et al. (2006). "Impact of agricultural inputs on soil organisms—a review." Soil Research 44(4): 379-406.

Bush, A. O. (2001). Parasitism: the diversity and ecology of animal parasites, Cambridge Univ Pr.

Butt, K. R. (1993). "Reproduction and growth of three deep-burrowing earthworms (Lumbricidae) in laboratory culture in order to assess production for soil restoration." Biology and Fertility of Soils 16(2): 135-138.

Butt, K. R. (2002). "Depth of cocoon deposition by three earthworm species in mesocosms." European Journal of Soil Biology 38(2): 151-153.

Butt, K. R., J. Frederickson, et al. (1999). "Colonisation, survival and spread of earthworms on a partially restored landfill site." Pedobiologia 43(6): 684-690.

Butt, K. R. and C. N. Lowe (2007). "Presence of earthworm species within and beneath Lumbricus terrestris (L.) middens." European Journal of Soil Biology 43: S57-S60.

C

Canales-Lazcano, J., J. Contreras-Garduno, et al. (2005). "Fitness-related attributes and gregarine burden in a non-territorial damselfly Enallagma praevarumhagen (Zygoptera: Coenagrionidae)." Odonatologica 34(2).

Capowiez, Y., S. Cadoux, et al. (2009). "The effect of tillage type and cropping system on earthworm communities, macroporosity and water infiltration." Soil and Tillage Research 105(2): 209-216.

Casemore, D. P. (1991). "Laboratory methods for diagnosing cryptosporidiosis." Journal of Clinical Pathology 44(6): 445-451.

Cator, L. J., K. R. Ng'Habi, et al. (2010). "Sizing up a mate: variation in production and response to acoustic signals in Anopheles gambiae." Behavioral Ecology 21(5): 1033-1039.

Chaine, A. and L. Angeloni (2005). "Size-dependent mating and gender choice in a simultaneous hermaphrodite, Bulla gouldiana." Behavioral Ecology and Sociobiology 59(1): 58-68.

Charlesworth, D. and B. Charlesworth (1987). "The effect of investment in attractive structures on allocation to male and female functions in plants." Evolution 41(5): 948-968.

Charmantier, A. and B. C. Sheldon (2006). "Testing genetic models of mate choice evolution in the wild." Trends in Ecology & Evolution 21(8): 417-419.

Charnov, E. L. (1979). "Simultaneous hermaphroditism and sexual selection." Proceedings of the National Academy of Sciences of the United States of America 76(5): 2480-2484.

Charnov, E. L. (1982). "The theory of sex allocation." Monographs in Population Biology: i-x, 1-355.

Charnov, E. L. (1987). "Sexuality and hermaphroditism in barnacles: A natural selection approach." Crustacean Issues 5: 89-103.

Charnov, E. L., J. J. Bull, et al. (1976). "Why be an hermaphrodite?" Nature 263(5573): 125-126.

Clapperton, J. M., J. J. Miller, et al. (1997). "Earthworm populations as affected by long-term tillage practices in southern Alberta, Canada." Soil Biology and Biochemistry 29(3-4): 631-633.

Clayton, D. H. (1991). "The influence of parasites on host sexual selection." Parasitology Today 7(12): 329-334.

Clutton-Brock, T. H. and G. A. Parker (1992). "Potential reproductive rates and the operation of sexual selection." Quarterly Review of Biology: 437-456.

Colin, P. L. (2010). "Aggregation and spawning of the humphead wrasse Cheilinus undulatus (Pisces: Labridae): General aspects of spawning behaviour." Journal of Fish Biology 76(4): 987-1007.

Collins, S. A., C. Hubbard, et al. (1994). "Female mate choice in the zebra finch - the effect of male beak color and male song." Behavioral Ecology and Sociobiology 35(1): 21-25.

Connallon, T. and A. G. Clark (2010). "Sex linkage, sex-specific selection, and the role of recombination in the evolution of sexually dimorphic gene expression." Evolution 64(12): 3417-3442.

Consuegra, S. and C. Garcia de Leaniz (2008). "MHC-mediated mate choice increases parasite resistance in salmon." Proceedings of the Royal Society B: Biological Sciences 275(1641): 1397-1403.

Cooper, E. L. and P. Roch (2003). "Earthworm immunity: A model of immune competence." Pedobiologia 47(5-6): 676-688.

Coq, S., B. G. Barthès, et al. (2007). "Earthworm activity affects soil aggregation and organic matter dynamics according to the quality and localization of crop residues--An experimental study (Madagascar)." Soil Biology and Biochemistry 39(8): 2119-2128.

Cosín, D. J. D., M. Novo, et al. (2011). Reproduction of earthworms: Sexual selection and parthenogenesis

Biology of Earthworms. A. Karaca, Springer Berlin Heidelberg. 24: 69-86.

Crow, J. F. (1994). "Advantages of sexual reproduction." Developmental Genetics 15(3): 205-213.

Cuendet, G. (1983). Predation on earthworms by the black-headed gull (Larus ridibundus L.), Chapman & Hall, London & New York.

Cunningham, E. J. A. and T. R. Birkhead (1998). "Sex roles and sexual selection." Animal Behaviour 56(6): 1311-1321.

Curry, J. P. (1998). "Factors affecting earthworm abundance in soils."

Curry, J. P., D. Byrne, et al. (2002). "Intensive cultivation can drastically reduce earthworm populations in arable land." European Journal of Soil Biology 38(2): 127-130.

D

Darwin, C. (1871). Part II, principals of sexual selection.

Darwin, C. (1874). The descent of man, and selection in relation to sex, J. Murray.

Decaëns, T. and J. J. Jiménez (2002). "Earthworm communities under an agricultural intensification gradient in Colombia." Plant and Soil 240(1): 133-143.

Decaestecker, E., S. Gaba, et al. (2007). "Host-parasite 'Red Queen' dynamics archived in pond sediment." Nature 450: 870-U816.

Devliegher, W. and W. Verstraete (1997). "The effect of Lumbricus terrestris on soil in relation to plant growth: Effects of nutrient-enrichment processes (NEP) and gut-associated processes (GAP)." Soil Biology & Biochemistry 29(3-4): 341-346.

DeWitt, T. J. (1996). "Gender contests in a simultaneous hermaphrodite snail: A size-advantage model for behaviour." Animal Behaviour 51: 345-351.

Dillen, L., K. Jordaens, et al. (2008). "Effects of isolation and body size on the mating behaviour of the hermaphroditic land snail Succinea putris." Animal Behaviour 75(4): 1401-1411.

Dillen, L., K. Jordaens, et al. (2010). "Effects of body size on courtship role, mating frequency and sperm transfer in the land snail Succinea putris." Animal Behaviour 79(5): 1125-1133.

Dobson, A., K. D. Lafferty, et al. (2008). "Homage to Linnaeus: How many parasites? How many hosts?" Proceedings of the National Academy of Sciences 105(Supplement 1): 11482-11489.

E

Eberhard, W. G. (1991). "Copulatory courtship and cryptic female choice in insects." Biological Reviews 66(1): 1-31.

Eberhard, W. G. (1996). Monographs in behavior and ecology: Female control: Sexual selection by cryptic female choice, Princeton University Press, Technical Books, 36 University Place, Princeton, New Jersey 08540, USA Oxford, England, UK.

Eberhard, W. G. and C. Cordero (1995). "Sexual selection by cryptic female choice on male seminal products - a new bridge between sexual selection and reproductive physiology." Trends in Ecology & Evolution 10(12): 493-496.

Ebert, D. (2008). "Host-parasite coevolution: Insights from the Daphnia-parasite model system." Current Opinion in Microbiology 11(3): 290-301.

Edwards, C. A., P. J. Bohlen, et al. (1995). Earthworms in agroecosystems, Lewis Publishers.

Ehlers, W. (1975). "Observations on earthworm channels and infiltration on tilled and untilled Loess soil." Soil Science 119(3): 242-249.

Eisenhauer, N., S. Marhan, et al. (2008). "Assessment of anecic behavior in selected earthworm species: Effects on wheat seed burial, seedling establishment, wheat growth and litter incorporation." Applied Soil Ecology 38(1): 79-82.

Elgar, M. A. (1992). Cannibalism : Ecology and evolution among diverse taxa. Oxford u.a., Oxford Univ. Press.

Endler, J. A. and A. L. Basolo (1998). "Sensory ecology, receiver biases and sexual selection." Trends in Ecology & Evolution 13(10): 415-420.

Eriksen-Hamel, N. S., A. B. Speratti, et al. (2009). "Earthworm populations and growth rates related to long-term crop residue and tillage management." Soil and Tillage Research 104(2): 311-316.

Eriksen-Hamel, N. S. and J. K. Whalen (2007). "Impacts of earthworms on soil nutrients and plant growth in soybean and maize agroecosystems." Agriculture Ecosystems & Environment 120(2-4): 442-448.

Ernst, G. and C. Emmerling (2009). "Impact of five different tillage systems on soil organic carbon content and the density, biomass, and community composition of earthworms after a ten year period." European Journal of Soil Biology 45(3): 247-251.

Escobar, J. S., J. R. Auld, et al. (2011). "Patterns of mating system evolution in hermaphroditic animals: Correlations among selfing rate, inbreeding depression, and the timing of reproduction." Evolution 65(5): 1233-1253.

Eshel, I. and W. D. Hamilton (1984). "Parent-offspring correlation in fitness under fluctuating selection." Proceedings of the Royal Society of London. Series B. Biological Sciences 222(1226): 1-14.

Estevez, B., A. Ndayegamiye, et al. (1996). "The effect on earthworm abundance and selected soil properties after 14 years of solid cattle manure and NPKMg fertilizer application." Canadian Journal of Soil Science 76(3): 351-355.

F

Farmer, J. N. (1980). The protozoa: An introduction to protozoology.

Field, S. G., J. Kurtz, et al. (2004). "Evaluation of an innate immune reaction to parasites in earthworms." Journal of Invertebrate Pathology 86(1-2): 45-49.

Field, S. G., M. Lange, et al. (2007). "Genetic diversity and parasite defense in a fragmented urban metapopulation of earthworms." Animal Conservation 10(2): 162-175.

Field, S. G. and N. K. Michiels (2005). "Parasitism and growth in the earthworm Lumbricus terrestris: fitness costs of the gregarine parasite Monocystis sp." Parasitology 130: 397-403.

Field, S. G. and N. K. Michiels (2006). "Acephaline gregarine parasites (Monocystis sp.) are not transmitted sexually among their lumbricid earthworm hosts." Journal of Parasitology 92(2): 292-297.

Field, S. G. and N. K. Michiels (2006). "Does the acephaline gregarine Monocystis sp modify the surface behaviour of its earthworm host Lumbricus terrestris?" Soil Biology & Biochemistry 38(6): 1334-1339.

Field, S. G., H. J. Schirp, et al. (2003). "The influence of Monocystis sp infection on growth and mating behaviour of the earthworm Lumbricus terrestris." Canadian Journal of Zoology-Revue Canadienne De Zoologie 81(7): 1161-1167.

Fisher, R. A. (1930). The genetical theory of natural selection, (Clarendon Press).

Folstad, I. and A. J. Karter (1992). "Parasites, bright males, and the immunocompetence handicap." American Naturalist: 603-622.

Franzluebbers, A. (2002). Conservation Tillage and Stratification of Soil Properties, Elsevier.

Freeland, W. J. (1976). "Pathogens and the evolution of primate sociality." Biotropica 8(1): 12-24.

G

Gallagher, A. V. and N. C. Wollenhaupt (1997). "Surface alfalfa residue removal by earthworms Lumbricus terrestris L. in a no-till agroecosystem." Soil Biology & Biochemistry 29(3-4): 477-479.

Gerhardt, H. C., S. D. Tanner, et al. (2000). "Female preference functions based on call duration in the gray tree frog (Hyla versicolor)." Behavioral Ecology 11(6): 663-669.

Gibson, R. M. (1996). "A re-evaluation of hotspot settlement in lekking sage grouse." Animal Behaviour 52: 993-1005.

Golding, D. W. and P. J. W. Olive (1978). "Patterns of regenerative growth, reproductive strategy, and endocrine control in polychaete annelids." Comparative Endocrinology: 117-120.

Gowaty, P. A. (2004). Sex roles, contests for the control of reproduction, and sexual selection, Cambridge University Press.

Greeff, J. M. and N. K. Michiels (1999a). "Sperm digestion and reciprocal sperm transfer can drive hermaphrodite sex allocation to equality." American Naturalist 153(4): 421-430.

Greeff, J. M. and N. K. Michiels (1999b). "Low potential for sexual selection in simultaneously hermaphroditic animals." Proceedings of the Royal

Society of London Series B-Biological Sciences 266(1429): 1671-1676.

Gregorius, H. R. and H. H. Hattemer (1987). "Charactarization of mating systems." Journal of Theoretical Biology 128(4): 407-422.

Grigoropoulou, N. and K. R. Butt (2010). "Field investigations of Lumbricus terrestris spatial distribution and dispersal through monitoring of manipulated, enclosed plots." Soil Biology and Biochemistry 42(1): 40-47.

Grigoropoulou, N., K. R. Butt, et al. (2008). "Effects of adult Lumbricus terrestris on cocoons and hatchlings in Evans' boxes." Pedobiologia 51(5): 343-349.

H

Haase, M. and A. Karlsson (2004). "Mate choice in a hermaphrodite: you won't score with a spermatophore." Animal Behaviour 67: 287-291.

Häderer, I., J. Werminghausen, et al. (2009). "No effect of mate novelty on sexual motivation in the freshwater snail Biomphalaria glabrata." Frontiers in Zoology 6(1): 23.

Haerty, W., E. Gentilhomme, et al. (2007). "Female preference for a male sexual trait uncorrelated with male body size in the Palmate newt (Triturus helveticus)." Behaviour 144: 797-814.

Hamilton, W. D. (1990). "Mate choice near or far." American Zoologist 30(2): 341-352.

Hamilton, W. D. and M. Zuk (1982). Heritable true fitness and bright birds: A role for parasites? 218: 384-387.

Hankison, S. J. and M. R. Morris (2003). "Avoiding a compromise between sexual selection and species recognition: Female swordtail fish assess multiple species-specific cues." Behavioral Ecology 14(2): 282-287.

Hansen, B., H. F. Alroe, et al. (2001). "Approaches to assess the environmental impact of organic farming with particular regard to Denmark." Agriculture Ecosystems & Environment 83(1-2): 11-26.

Hecker, K. R., M. R. Forbes, et al. (2002). "Parasitism of damselflies (Enallagma boreale) by gregarines: Sex biases and relations to adult survivorship." Canadian journal of zoology 80(1): 162-168.

Hendrix, P. F. (1998). "Earthworms in agroecosystems: A summary of current research."

Hermann, P. M., B. Genereux, et al. (2009). "Evidence for age-dependent mating strategies in the simultaneous hermaphrodite snail, Lymnaea stagnalis (L.)." Journal of Experimental Biology 212(19): 3164-3173.

Hess, W. N. (1925). "Photoreceptors of Lumbricus terrestris, with special reference to their distribution, structure, and function." Journal of Morphology 41(1): 63-93.

Hill, G. E. (1990). "Female house finches prefer colorful males - sexual selection for a condition-dependent trait." Animal Behaviour 40: 563-572.

Hill, G. E. (1991). "Plumage coloration is a sexually selected indicator of male quality." Nature 350(6316): 337-339.

Hine, E., S. F. Chenoweth, et al. (2004). "Multivariate quantitative genetics and the lek paradox: Genetic variance in male sexually selected traits of Drosophila serrata under field conditions." Evolution 58(12): 2754-2762.

Hoffer, J., J. Ellers, et al. (2010). "Costs of receipt and donation of ejaculates in a simultaneous hermaphrodite." Bmc Evolutionary Biology 10(1): 393.

Hole, D. G., A. J. Perkins, et al. (2005). "Does organic farming benefit biodiversity?" Biological conservation 122(1): 113-130.

Holland, J. M. (2004). "The environmental consequences of adopting conservation tillage in Europe: Reviewing the evidence." Agriculture, Ecosystems & Environment 103(1): 1-25.

Holm, S. (1979). "A simple sequentially rejective multiple test procedure." Scandinavian Journal of Statistics 6(2): 65-70.

Hosken, D. J. (2009). "The evolutionary origin and maintenance of sperm: Selection for a small, motile gamete mating type." Sperm biology: an evolutionary perspective: 43.

Howard, R. S. and C. M. Lively (2003). "Opposites attract? Mate choice for parasite evasion and the evolutionary stability of sex." Journal of Evolutionary Biology 16(4): 681-689.

Hunt, J., C. J. Breuker, et al. (2009). "Male–male competition, female mate choice and their interaction: Determining total sexual selection." Journal of Evolutionary Biology 22(1): 13-26.

I/J

Iyengar, V. K., C. Rossini, et al. (2001). "Precopulatory assessment of male quality in an arctiid moth (Utetheisa ornatrix): Hydroxydanaidal is the only criterion of choice." Behavioral Ecology and Sociobiology 49(4): 283-288.

Iyer, P. and J. Roughgarden (2008). "Dioecy as a specialization promoting sperm delivery." Evolutionary Ecology Research 10(6): 867-892.

Iyer, P. and J. Roughgarden (2008). "Gametic conflict versus contact in the evolution of anisogamy." Theoretical Population Biology 73(4): 461-472.

Jacobs, A. C. and M. Zuk (2011). "Sexual selection and parasites." Eco-Immunology: 468.

James, M. C., S. A. Eckert, et al. (2005). "Migratory and reproductive movements of male leatherback turtles (Dermochelys coriacea)." Marine Biology 147(4): 845-853.

Jamieson, B. G. M., T. P. Fleming, et al. (1982). "An ultrastructural study of spermatogenesis and spermatozoal morphology in Sparganophilus tamesis (Sparganophilidae, Oligochaeta, Annelida)." J Zool 196: 63-79.

Janicke, T., H. Kesselring, et al. (2012). "Strategic mating effort in a simultaneous hermaphrodite." Behavioral Ecology and Sociobiology: 1-9.

Jarne, P. and J. R. Auld (2006). "Animals mix it up too: The distribution of self-fertilization among hermaphroditic animals." Evolution 60(9): 1816-1824.

Jensen, K. H., T. Little, et al. (2006). "Empirical support for optimal virulence in a castrating parasite." Plos Biology 4(7): 1265-1269.

Johnson-Maynard, J. L., K. J. Umiker, et al. (2007). "Earthworm dynamics and soil physical properties in the first three years of no-till management." Soil and Tillage Research 94(2): 338-345.

Johnson-Maynard, J. L., K. J. Umiker, et al. (2007). "Earthworm dynamics and soil physical properties in the first three years of no-till management." Soil & Tillage Research 94(2): 338-345.

Johnstone, R. A. (1996). "Multiple displays in animal communication:`Backup signals' and `Multiple messages'." Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 351(1337): 329-338.

Jordaens, K., J. Pinceel, et al. (2005). "Mate choice in the hermaphroditic land snail Succinea putris (Stylommatophora : Succineidae)." Animal Behaviour 70: 329-337.

Jordan, D., R. J. Miles, et al. (2004). "Effect of management practices and cropping systems on earthworm abundance and microbial activity in Sanborn Field: a 115-year-old agricultural field." Pedobiologia 48(2): 99-110.

K

Kay, B. D. and L. J. Munkholm (2011). "Managing the interactions between soil biota and their physical habitat in agroecosystems." Architecture and Biology and Soils: Life in Inner Space: 170.

Keller, L. F. and H. K. Reeve (1995). Why do females mate with multiple males? The sexually selected sperm hypothesis. Advances in the Study of Behavior. J. S. R. C. T. S. Peter J.B. Slater and M. Manfred, Academic Press. Volume 24: 291-315.

Kennedy, C. E. J., J. A. Endler, et al. (1987). "Parasite load predicts mate choice in guppys." Behavioral Ecology and Sociobiology 21(5): 291-295.

Kirkpatrick, M. and M. J. Ryan (1991). "The evolution of mating preferences and the paradox of the lek." Nature 350(6313): 33-38.

Kleemann, G. A. and A. L. Basolo (2007). "Facultative decrease in mating resistance in hermaphroditic Caenorhabditis elegans with self-sperm depletion." Animal Behaviour 74: 1339-1347.

Klingenberg, C. P., R. H. B. Leigh, et al. (1997). "Influence of gut parasites on growth performance in the water strider Gerris buenoi (Hemiptera: Gerridae)." Ecography 20(1): 29-36.

Koene, J. M., R. F. Jansen, et al. (2000). "A conserved location for the central nervous system control of mating behaviour in gastropod molluscs: Evidence from a terrestrial snail." Journal of Experimental Biology 203(6): 1071-1080.

Koene, J. M., K. Montagne-Wajer, et al. (2007). "Aspects of body size and mate choice in the simultaneously hermaphroditic pond snail Lymnaea stagnalis." Animal Biology 57(2): 247-259.

Koene, J. M., T. Pförtner, et al. (2005). "Piercing the partner's skin influences sperm uptake in the earthworm Lumbricus terrestris." Behavioral Ecology and Sociobiology 59(2): 243-249.

Koene, J. M. and H. Schulenburg (2005). "Shooting darts: Co-evolution and counter-adaptation in hermaphroditic snails." Bmc Evolutionary Biology 5(1): 25.

Koene, J. M., G. Sundermann, et al. (2002). "On the function of body piercing during copulation in earthworms." Invertebrate Reproduction & Development 41(1-3): 35-40.

Koene, J. M. and A. Ter Maat (2005). "Sex role alternation in the simultaneously hermaphroditic pond snail Lymnaea stagnalis is determined by the availability of seminal fluid." Animal Behaviour 69(4): 845-850.

Koepke, U. (2003). Conservation agriculture with and without use of agrochemicals.

Kokko, H. (2001). "Fisherian and “good genes” benefits of mate choice: How (not) to distinguish between them." Ecology Letters 4(4): 322-326.

Kokko, H., M. D. Jennions, et al. (2006). "Unifying and testing models of sexual selection." Annual Review of Ecology Evolution and Systematics 37: 43-66.

Kokko, H. and R. A. Johnstone (2002). "Why is mutual mate choice not the norm? Operational sex ratios, sex roles and the evolution of sexually dimorphic and monomorphic signalling." Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 357(1419): 319-330.

Kokko, H. and P. Monaghan (2001). "Predicting the direction of sexual selection." Ecology Letters 4(2): 159-165.

Kose, M. and A. P. Møller (1999). "Sexual selection, feather breakage and parasites: The importance of white spots in the tail of the barn swallow (Hirundo rustica)." Behavioral Ecology and Sociobiology 45(6): 430-436.

Kotiaho, J. S., N. R. LeBas, et al. (2008). "On the resolution of the lek paradox." Trends in Ecology & Evolution 23(1): 1-3.

Kozlowski, J. and S. C. Stearns (1989). "Hypotheses for the production of excess zygotes: Models of bet-hedging and selective abortion." Evolution 43(7): 1369-1377.

Krupp, J. J., C. Kent, et al. (2008). "Social experience modifies pheromone expression and mating behavior in male Drosophila melanogaster." Current Biology 18(18): 1373-1383.

L

Lachnicht, S. L., R. W. Parmelee, et al. (1997). "Characteristics of macroporosity in a reduced tillage agroecosystem with manipulated earthworm populations: Implications for infiltration and nutrient transport." Soil Biology & Biochemistry 29(3-4): 493-498.

Lal, R., D. L. Reicosky, et al. (2007). "Evolution of the plow over 10,000 years and the rationale for no-till farming." Soil & Tillage Research 93(1): 1-12.

Lancaster, L. T., C. A. Hipsley, et al. (2009). "Female choice for optimal combinations of multiple male display traits increases offspring survival." Behavioral Ecology 20(5): 993-999.

Landry, C., D. Garant, et al. (2001). "'Good genes as heterozygosity': The major histocompatibility complex and mate choice in Atlantic salmon

(Salmo salar)." Proceedings of the Royal Society of London. Series B: Biological Sciences 268(1473): 1279-1285.

Langmaack, M. (1999). "Earthworm communities in arable land influenced by tillage, compaction, and soil." Z. Kol. Natursch 8: 11-21.

Lavelle, P., D. Bignell, et al. (1997). "Soil function in a changing world: the role of invertebrate ecosystem engineers." European Journal of Soil Biology 33(4): 159-193.

Leander, B. S., R. E. Clopton, et al. (2003). "Phylogeny of gregarines (Apicomplexa) as inferred from small-subunit rDNA and β-tubulin." International Journal of Systematic and Evolutionary Microbiology 53(1): 345-354.

Lee, K. E. (1983). Earthworms of tropical regions - some aspects of their ecology and relationships with soils, Chapman & Hall, London & New York.

Lee, K. E. (1985). Earthworms: Their ecology and relationships with soils and land use, Academic Press, Sydney, Orlando etc.

Lehmann, L., L. F. Keller, et al. (2007). "Mate choice evolution, dominance effects, and the maintenance of genetic variation." Journal of Theoretical Biology 244(2): 282-295.

Lehtonen, J. and H. Kokko (2011). "Two roads to two sexes: Unifying gamete competition and gamete limitation in a single model of anisogamy evolution." Behavioral Ecology and Sociobiology 65(3): 445-459.

Leonard, J. L. (2005). "Bateman's principle and simultaneous hermaphrodites: A paradox." Integrative and Comparative Biology 45(5): 856-873.

Leonard, J. L. (2006). "Sexual selection: Lessons from hermaphrodite mating systems." Integr. Comp. Biol. 46(4): 349-367.

Leonard, J. L. and K. Lukowiak (1991). "Sex and the simultaneous hermaphrodite: Testing models of male-female conflict in a sea slug, Navanax intermis (Opisthobranchia)." Animal Behaviour 41(2): 255-266.

Lindahl, A. M. L., I. G. Dubus, et al. (2009). "Site classification to predict the abundance of the deep-burrowing earthworm Lumbricus terrestris L." Vadose Zone Journal 8(4): 911-915.

Lively, C. M. and M. F. Dybdahl (2000). "Parasite adaptation to locally common host genotypes." Nature 405(6787): 679-681.

Lohse, K., A. Gutierrez, et al. (2006). "Experimental evolution of resistance in Paramecium caudatum against the bacterial parasite Holospora undulata." Evolution 60(6): 1177-1186.

Loose, M. J. and J. M. Koene (2008). "Sperm transfer is affected by mating history in the simultaneously hermaphroditic snail Lymnaea stagnalis." Invertebrate Biology 127(2): 162-167.

Lorch, P. D., S. Proulx, et al. (2003). "Condition-dependent sexual selection can accelerate adaptation." Evolutionary Ecology Research 5(6): 867-881.

Lüscher, A. and C. Wedekind (2002). "Size-dependent discrimination of mating partners in the simultaneous hermaphroditic cestode Schistocephalus solidus." Behav. Ecol. 13(2): 254-259.

M

Marden, J. H. and J. R. Cobb (2004). "Territorial and mating success of dragonflies that vary in muscle power output and presence of gregarine gut parasites." Animal Behaviour 68(4): 857-865.

Mathieu, J., S. Barot, et al. (2010). "Habitat quality, conspecific density, and habitat pre-use affect the dispersal behaviour of two earthworm species, Aporrectodea icterica and Dendrobaena veneta, in a mesocosm experiment." Soil Biology and Biochemistry 42(2): 203-209.

Mays Jr, H. L. and G. E. Hill (2004). "Choosing mates: Good genes versus genes that are a good fit." Trends in Ecology & Evolution 19(10): 554-559.

McCarthy, T. M. and A. Sih (2008). "Relatedness of mates influences mating behaviour and reproductive success of the hermaphroditic freshwater snail Physa gyrina." Evolutionary Ecology Research 10(1): 77-94.

Meirmans, P. G. and P. H. Van Tienderen (2004). "GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of

asexual organisms." Molecular Ecology Notes 4: 792-794.

Mele, P. M. and M. R. Carter (1999). "Impact of crop management factors in conservation tillage farming on earthworm density, age structure and species abundance in south-eastern Australia." Soil and Tillage Research 50(1): 1-10.

Michiels, N. K. (1998). "Mating conflicts and sperm competition in simultaneous hermaphrodites." Sperm competition and sexual selection: 219.

Michiels, N. K. (1999). "Sexual adaptations to high density in hermaphrodites." Invertebrate Reproduction & Development 36(1-3): 35-40.

Michiels, N. K. and B. Bakovski (2000). "Sperm trading in a hermaphroditic flatworm: Reluctant fathers and sexy mothers." Animal Behaviour 59(2): 319-325.

Michiels, N. K., A. Hohner, et al. (2001). "Precopulatory mate assessment in relation to body size in the earthworm Lumbricus terrestris: Avoidance of dangerous liaisons?" Behavioral Ecology 12(5): 612-618.

Michiels, N. K. and J. M. Koene (2006). "Sexual selection favors harmful mating in hermaphrodites more than in gonochorists." Integrative and Comparative Biology 46(4): 473-480.

Michiels, N. K. and A. Kuhl (2003). "Altruistic sperm donation in a sperm-dependent parthenogenetic hermaphrodite is stabilized by reciprocal sperm exchange." Journal of Zoology 259: 77-82.

Michiels, N. K. and L. J. Newman (1998). "Sex and violence in hermaphrodites." Nature 391(6668): 647-647.

Milinski, M. and T. C. M. Bakker (1990). "Female stickbacks use male coloration in mate choice and hence avoid parasitized males." Nature 344(6264): 330-333.

Minoretti, N., D. Schmera, et al. (2011). "Determinants of female and male reproductive success in a simultaneous hermaphrodite land snail." Animal Behaviour.

Møller, A. P. (1990). "Fluctuating asymmetry in male sexual ornaments may reliably reveal male quality." Animal Behaviour 40: 1185-1187.

Møller, A. P. (1992). "Parasites differentially increase the degree of fluctuating asymmetry in secondary sexual characters." Journal of Evolutionary Biology 5(4): 691-699.

Møller, A. P. and A. Pomiankowski (1993). "Why have birds got multiple sexual ornaments?" Behavioral Ecology and Sociobiology 32(3): 167-176.

Møller, A. P. and N. Saino (1994). "Parasites, immunology of hosts, and host sexual selection." The Journal of parasitology: 850-858.

Monroy, F., M. Aira, et al. (2005). "Size-assortative mating in the earthworm Eisenia fetida (Oligochaeta, Lumbricidae)." Journal of Ethology 23(1): 69-70.

Morgan, M. T. (1994). "Models of sexual selection in hermaphrodites, especially plants." The American Naturalist 144(ArticleType: research-article / Issue Title: Supplement: Sexual Selection in Plants and Animals / Full publication date: Aug., 1994 / Copyright © 1994 The University of Chicago Press): S100-S125.

Morgan, T. (1994). "Models of Sexual Selection in Hermaphrodites, Especially Plants." The American Naturalist Vol. 144: 100-125.

Munday, P. L., P. M. Buston, et al. (2006). "Diversity and flexibility of sex-change strategies in animals." Trends in Ecology & Evolution 21(2): 89-95.

N

Neff, B. D. and T. E. Pitcher (2005). "Genetic quality and sexual selection: An integrated framework for good genes and compatible genes." Molecular Ecology 14(1): 19-38.

Novo, M., A. Almodóvar, et al. (2010). "Mate choice of an endogeic earthworm revealed by microsatellite markers." Pedobiologia 53(6): 375-379.

Nuutinen, V. and K. R. Butt (1997). "The mating behaviour of the earthworm Lumbricus terrestris (Oligochaeta: Lumbricidae)." Journal of Zoology (London) 242(4): 783-798.

Nuutinen, V. and K. R. Butt (1997). "Pre-mating behaviour of the earthworm Lumbricus terrestris L." Soil Biology & Biochemistry 29(3-4): 307-308.

Nuutinen, V. and K. R. Butt (2005). "Homing ability widens the sphere of influence of the earthworm Lumbricus terrestris L." Soil Biology and Biochemistry 37(4): 805-807.

O

O'Donoghue, P. J. (1995). "Cryptosporidium and cryptosporidiosis in man and animals." International Journal for Parasitology 25(2): 139-195.

Oldenburg, E., S. Kramer, et al. (2008). "Impact of the earthworm Lumbricus terrestris on the degradation of Fusarium-infected and deoxynivalenol-contaminated wheat straw." Soil Biology and Biochemistry 40(12): 3049-3053.

Otsuka, C., Y. Rouger, et al. (1980). "A possible relationship between size and reproductive behavior in a population of Aplysia punctata (Cuvier 1803)." Veliger 23(2): 159-162.

Oyedele, D. J., P. Schjønning, et al. (2006). "Physicochemical properties of earthworm casts and uningested parent soil from selected sites in southwestern Nigeria." Ecological Engineering 28(2): 106-113.

P

Pal, P., J. Erlandsson, et al. (2006). "Size-assortative mating and non-reciprocal copulation in a hermaphroditic intertidal limpet: Test of the mate availability hypothesis." Marine Biology 148(6): 1273-1282.

Paoletti, M. G. (1999). "The role of earthworms for assessment of sustainability and as bioindicators." Agriculture Ecosystems & Environment 74(1-3): 137-155.

Parker, G. A. (1970). "Sperm competition and its evolutionary consequences in the insects." Biological Reviews 45(4): 525-567.

Parker, G. A., R. R. Baker, et al. (1972). "The origin and evolution of gamete dimorphism and the male-female phenomenon." Journal of Theoretical Biology 36(3): 529-553.

Parker, G. A. and T. Pizzari (2010). "Sperm competition and ejaculate economics." Biological Reviews 85(4): 897-934.

Peigné, J., M. Cannavaciuolo, et al. (2009). "Earthworm populations under different tillage systems in organic farming." Soil and Tillage Research 104(2): 207-214.

Pélissié, B., P. Jarne, et al. (2012). "Sexual selection without sexual dimorphism: Bateman gradients in a simultaneous hermaphrodite." Evolution 66(1): 66-81.

Pelosi, C., M. Bertrand, et al. (2009). "Earthworm community in conventional, organic and direct seeding with living mulch cropping systems." Agronomy for sustainable development 29(2): 287-295.

Peters, A. and N. K. Michiels (1996). "Do simultaneous hermaphrodites choose their mates? Effects of body size in a planarian flatworm." Freshwater Biology 36(3): 623-630.

Pfiffner, L. (1993). "Long term effects of biological and conventional farming on earthworm populations." Zeitschrift Fur Pflanzenernahrung Und Bodenkunde 156(3): 259-265.

Pfiffner, L. and H. Luka (2007). "Earthworm populations in two low-input cereal farming systems." Applied Soil Ecology 37(3): 184-191.

Pitkänen, J. and V. Nuutinen (1997). "Distribution and abundance of burrows formed by Lumbricus terrestris L. and Aporrectodea caliginosa Sav. in the soil profile." Soil Biology and Biochemistry 29(3–4): 463-467.

Pizl, V. (1992). "Succession of earthworm populations in abandoned fields." Soil Biology and Biochemistry 24(12): 1623-1628.

Pizzari, T. and R. R. Snook (2003). "Perspective: Sexual conflict and sexual selection: Chasing away paradigm shifts." Evolution 57(6): 1223-1236.

Pongratz, N. and N. K. Michiels (2003). "High multiple paternity and low last-male sperm precedence in a hermaphroditic planarian flatworm: Consequences for reciprocity patterns." Molecular Ecology 12(6): 1425-1433.

Poulin, R. and W. L. Vickery (1996). "Parasite mediated sexual selection: Just how choosy are parasitized females?" Behavioral Ecology and Sociobiology 38(1): 43-49.

Poullain, V., S. Gandon, et al. (2008). "The evolution of specificity in evolving and coevolving antagonistic interactions between a bacteria and its phage." Evolution 62(1): 1-11.

Protz, R., C. A. Fox, et al. (2000). "Estimating earthworm-influenced soil structure by morphometric image analysis." Soil Science Society of America Journal 64(3): 982-988.

Pryke, S. R., S. Andersson, et al. (2001). "Sexual selection of multiple handicaps in the red-collared widowbird: Female choice of tail length but not carotenoid display." Evolution 55(7): 1452-1463.

Pryke, S. R., L. A. Rollins, et al. (2010). "Females use multiple mating and genetically loaded sperm competition to target compatible genes." Science 329(5994): 964-967.

Pusey, A. and M. Wolf (1996). "Inbreeding avoidance in animals." Trends in Ecology & Evolution 11(5): 201-206.

Puurtinen, M., T. Ketola, et al. (2009). "The good-genes and compatible-genes benefits of mate choice." The American Naturalist 174(5): 741-752.

R

Reinhart, M. and N. Dollahon (2003). "Responses of coelomocytes from Lumbricus terrestris to native and non-native eukaryotic parasites: The 7th international symposium on earthworm ecology · Cardiff · Wales · 2002." Pedobiologia 47(5-6): 710-716.

Rizhiya, E., C. Bertora, et al. (2007). "Earthworm activity as a determinant for N2O emission from crop residue." Soil Biology and Biochemistry 39(8): 2058-2069.

Rodriguez-Castellanos, L. and J. C. Sanchez-Hernandez (2007). "Earthworm biomarkers of pesticide contamination: Current status and perspectives." Journal of Pesticide Science 32(4): 360-371.

Rodriguez, Y., C. K. Omoto, et al. (2007). "Individual and population effects of Eugregarine, Gregarina niphandrodes (Eugregarinida: Gregarinidae), on Tenebrio molitor (Coleoptera: Tenebrionidae)." Environmental entomology 36(4): 689-693.

Rolff, J. and M. T. Siva-Jothy (2003). "Invertebrate Ecological Immunology." Science 301(5632): 472-475.

Rowe, L. and D. Houle (1996). "The lek paradox and the capture of genetic variance by condition dependent traits." Proceedings: Biological Sciences 263(1375): 1415-1421.

Rundle, H. D. and D. Schluter (1998). "Reinforcement of stickleback mate preferences: Sympatry breeds contempt." Evolution 52(1): 200-208.

Ryan, M. J. (1980). "Female mate choice in a neotropical frog Physalaemus pustulosus." Science (Washington D C) 209(4455): 523-525.

S

Sahm, S., T. P. Velavan, et al. (2009). "Reconstruction of mating history–a retrospective analysis of Lumbricus terrestris mate choice criteria in natural populations." Host–Parasite Interactions: 43.

Sakaluk, S. K. (2000). "Sensory exploitation as an evolutionary origin to nuptial food gifts in insects." Proceedings of the Royal Society of London. Series B: Biological Sciences 267(1441): 339-343.

Schärer, L. and T. Janicke (2009). "Sex allocation and sexual conflict in simultaneously hermaphroditic animals." Biology Letters 5(5): 705-708.

Schärer, L., L. M. Karlsson, et al. (2001). "Size dependent sex allocation in a simultaneous hermaphrodite parasite." Journal of Evolutionary Biology 14(1): 55-67.

Schärer, L., L. Rowe, et al. (2012). "Anisogamy, chance and the evolution of sex roles." Trends in Ecology & Evolution(0).

Schärer, L., P. Sandner, et al. (2005). "Trade-off between male and female allocation in the simultaneously hermaphroditic flatworm Macrostomum sp." Journal of Evolutionary Biology 18(2): 396-404.

Scheu, S. (2003). "Effects of earthworms on plant growth: Patterns and perspectives." Pedobiologia 47(5-6): 846-856.

Scheu, S. (2003). "Effects of earthworms on plant growth: patterns and perspectives: The 7th international symposium on earthworm ecology · Cardiff · Wales · 2002." Pedobiologia 47(5–6): 846-856.

Scheu, S., N. Schlitt, et al. (2002). "Effects of the presence and community composition of earthworms on microbial community functioning." Oecologia 133(2): 254-260.

Scheu, S. and H. Setala (2002). Multitrophic interactions in decomposer food-webs, Cambridge University Press; Cambridge University Press.

Schjorring, S. and I. Jager (2007). "Incestuous mate preference by a simultaneous hermaphrodite with strong inbreeding depression." Evolution 61(2): 423-430.

Schmeller, D. S., R. O'Hara, et al. (2005). "Male adaptive stupidity: Male mating pattern in hybridogenetic frogs." Evolutionary Ecology Research 7(7): 1039-1050.

Schmidt, G. D. and S. R. Roberts (2005). Foundations of Parasitology, McGraw-Hill Publishing.

Schmidt, W., O. Nitzsche, et al. (2003). "Implementation of conservation tillage as erosion control strategy on cropland in Saxony." Saxon State Agency for Agriculture, Leipzig, Germany. 9pp.

Schmitt, V., N. Anthes, et al. (2007). "Mating behaviour in the sea slug Elysia timida (Opisthobranchia, Sacoglossa): Hypodermic injection, sperm transfer and balanced reciprocity." Front Zool 4: 17.

Shackleton, M. A., M. D. Jennions, et al. (2005). "Fighting success and attractiveness as predictors of male mating success in the black field cricket Teleogryllus commodus: The effectiveness of no-choice tests." Behavioral Ecology and Sociobiology 58(1): 1-8.

Shaw, K. L. (2000). "Interspecific genetics of mate recognition: Inheritance of female acoustic preference in Hawaiian crickets." Evolution 54(4): 1303-1312.

Shipitalo, M. J. and K. R. Butt (1999). "Occupancy and geometrical properties of Lumbricus terrestris L. - burrows affecting infiltration." Pedobiologia 43(6): 782-794.

Shipitalo, M. J. and R.-C. Le Bayon (2004). Quantifying the effects of earthworms on soil aggregation and porosity, CRC Press.

Shuster, W. D. and C. A. Edwards (2003). "Interactions between tillage and earthworms in agroecosystems." Soil Tillage in Agroecosystems. CRC Press, Boca Raton, Florida, USA: 229-260.

Simmons, L. W. and B. Roberts (2005). "Bacterial immunity traded for sperm viability in male crickets." Science 309(5743): 2031.

Sims and Gerard (1985). Earthworms. Synopses of the British fauna, Linnean Society of London and Estuarine and Brachish-Water-Sciences Association.

Snyder, B. F. and P. A. Gowaty (2007). "A reappraisal of Bateman´s classic study of intrasexual selection." Evolution 61(11): 2457-2468.

Sprenger, D., J. Faber, et al. (2008). "Natural female mating rate maximizes hatchling size in a marine invertebrate." Journal of Animal Ecology 77(4): 696-701.

Sprenger, D., R. Lange, et al. (2011). "Population density and group size effects on reproductive behavior in a simultaneous hermaphrodite." Bmc Evolutionary Biology 11(1): 107.

Sprenger, D., R. Lange, et al. (2009). "The role of body size in early mating behavior in a simultaneous hermaphrodite, Chelidonura sandrana." Behavioral Ecology and Sociobiology 63(6): 953-958.

Stiernagel, T. (2006). "Maintencance of C. elegans." WormBook: 1-11.

Stolze, M., A. Piorr, et al., Eds. (2000). Environmental impacts of organic farming in Europe.

Strudley, M. W., T. R. Green, et al. (2008). "Tillage effects on soil hydraulic properties in space and time: State of the science." Soil and Tillage Research 99(1): 4-48.

Szilágyi, A., I. Scheuring, et al. (2009). "The evolution of intermediate castration virulence and ant coexistence in a spatially structured environment." Ecology Letters 12(12): 1306-1316.

T

Tato, A., A. Velando, et al. (2006). "Influence of size and partner preference on the female function of the earthworm Eisenia andrei (Oligochaeta, Lumbricidae)." European Journal of Soil Biology 42: S331-S333.

Taylor, P. D. and G. C. Williams (1982). "The lek paradox is not resolved." Theoretical Population Biology 22(3): 392-409.

Tebrügge, F. and R. A. Düring (1999). "Reducing tillage intensity—a review of results from a long-term study in Germany." Soil and Tillage Research 53(1): 15-28.

Thornhill, R. (1980). "Rape in Panorpa scorpionflies and a general rape hypothesis." Animal Behaviour 28(1): 52-59.

Thornhill, R. (1983). "Cryptic female choice and its implications in the scorpionfly Harpobittacus nigriceps." American Naturalist 122(6): 765-788.

Thornhill, R. and S. W. Gangestad (2006). "Facial sexual dimorphism, developmental stability, and susceptibility to disease in men and women." Evolution and Human Behavior 27(2): 131-144.

Tomiyama, K. (1996). "Mate-choice criteria in a protandrous simultaneously hermaphroditic land snail, Achatina fulica (Ferussac) (Stylommatophora, Achatinidae)." J. Mollus. Stud. 62(1): 101-111.

Tomkins, J. L., J. Radwan, et al. (2004). "Genic capture and resolving the lek paradox." Trends in Ecology & Evolution 19(6): 323-328.

Tregenza, T. and N. Wedell (2002). "Polyandrous females avoid costs of inbreeding." Nature 415(6867): 71-73.

Triplett Jr, G. B. and W. A. Dick (2008). "No-tillage crop production: a revolution in agriculture!" Agronomy Journal 100(3): 153.

V

Valembois, P., M. Lassegues, et al. (1992). "Formation of brown bodies in the coelomic cavity of the earthworm Eisenia foetida-andrei and attendant changes in shape and adhesive capacity of constitutive cells." Developmental and Comparative Immunology 16(2-3): 95-101.

Valembois, P., J. Seymour, et al. (1994). "Evidence of lipofucsin and melanin in the brown body of the earthworm Eisenia fetida-abndrei." Cell and Tissue Research 277(1): 183-188.

Van Valen, L. (1977). "The Red Queen." American Naturalist 111(980): 809.

Velando, A., J. Eiroa, et al. (2008). "Brainless but not clueless: Earthworms boost their ejaculates when they detect fecund non-virgin partners." Proceedings of the Royal Society B-Biological Sciences 275(1638): 1067-1072.

Velavan, T. P., H. Schulenburg, et al. (2007). "Development and characterization of novel microsatellite markers for the common earthworm (Lumbricus terrestris L.)." Molecular Ecology Notes 7(6): 1060-1062.

Velavan, T. P., H. Schulenburg, et al. (2010). "Detection of multiple infections by Monocystis strains in a single earthworm host using ribosomal internal transcribed spacer sequence variation." Parasitology 137(01): 45-51.

Velavan, T. P., S. Weller, et al. (2009). "High genetic diversity and heterogeneous parasite load in the earthworm Lumbricus terrestris on a German meadow." Soil Biology and Biochemistry In Press, Corrected Proof.

Verburgt, L., M. Ferreira, et al. (2011). "Male field cricket song reflects age, allowing females to prefer young males." Animal Behaviour 81(1): 19-29.

Vizoso, D. B., G. Rieger, et al. (2010). "Goings-on inside a worm: Functional hypotheses derived from sexual conflict thinking." Biological Journal of the Linnean Society 99(2): 370-383.

Vreys, C. and N. K. Michiels (1997). "Flatworms flatten to size up each other." Proceedings of the Royal Society of London Series B-Biological Sciences 264(1388): 1559-1564.

Vreys, C. and N. K. Michiels (1998). "Sperm trading by volume in a hermaphroditic flatworm with mutual penis intromission." Animal Behaviour 56(3): 777-785.

Vreys, C., E. R. Schockaert, et al. (1997). "Unusual pre-copulatory behaviour in the hermaphroditic Planarian glatworm, Dugesia gonocephala (Tricladida, Paludicola)." Ethology 103(3): 208-221.

W

Wagenaar, D. A., M. S. Hamilton, et al. (2010). "A hormone-activated central pattern generator for courtship." Current Biology 20(6): 487-495.

Wallwork, J. A. (1983). Earthworm biology, E. Arnold (Publishers) Ltd.

Warner, R. R. (1975). "The adaptive significance of sequential hermaphroditism in animals." The American Naturalist 109(965): 61-82.

Warner, R. R. (1995). "Large mating aggregations and daily long-distance spawning migrations in the bluehead wrasse, Thalassoma bifasciatum." Environmental Biology of Fishes 44(4): 337-345.

Weatherhead, P. J. and R. J. Robertson (1979). "Offspring quality and the polygyny threshold - sexy son hypothesis." American Naturalist 113(2): 201-208.

Webster, J. P. and C. M. Gower (2006). "Mate choice, frequency dependence, and the maintenance of resistance to parasitism in a simultaneous hermaphrodite." Integrative and Comparative Biology 46(4): 407-418.

Webster, J. P., J. I. Hoffman, et al. (2003). "Parasite infection, host resistance and mate choice: Battle of the genders in a simultaneous hermaphrodite." Proceedings of the Royal Society of London Series B-Biological Sciences 270(1523): 1481-1485.

Wedekind, C. (1992). "Detailed information about parasites revealed by sexual ornamentation." Proceedings: Biological Sciences 247(1320): 169-174.

Wedekind, C. (1994). "Mate choice and maternal selection for specific parasite resistances before, during and after fertilization." Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 346(1317): 303-311.

Wedekind, C., T. Seebeck, et al. (1995). "MHC-dependent mate preferences in humans." Proceedings: Biological Sciences 260(1359): 245-249.

Wedell, N. (1991). "Sperm competition selects for nuptial feeding in a bushcricket." Evolution 45(8): 1975-1978.

Willis, M. A. and M. C. Birch (1982). "Male lek formation and female calling in a population of the arctiid moth Estigmene acrea." Science (Washington D C) 218(4568): 168-170.

Wyss, E. and M. Glasstetter (1992). "Tillage treatments and earthworm distribution in a Swiss experimental corn field." Soil Biology and Biochemistry 24(12): 1635-1639.

X/Y/Z

Yang, J. N. (2010). "Cooperation and the evolution of anisogamy." Journal of Theoretical Biology 264(1): 24-36.

Zeh, D. W. and R. L. Smith (1985). "Paternal investment by terrestrial arthropods." American Zoologist 25(3): 785-805.

Zeh, J. A. and D. W. Zeh (1996). "The evolution of polyandry I: Intragenomic conflict and genetic incompatibility." Proceedings of the Royal Society of London. Series B: Biological Sciences 263(1377): 1711-1717.

Zeh, J. A. and D. W. Zeh (1997). "The evolution of polyandry II: Post–copulatory defenses against genetic incompatibility." Proceedings of the Royal Society of London. Series B: Biological Sciences 264(1378): 69-75.

Zhang, H. and S. Schrader (1993). "Earthworm effects on selected physical and chemical properties of soil aggregates." Biology and Fertility of Soils 15(3): 229-234.

Zieler, H. and J. A. Dvorak (2000). "Invasion in vitro of mosquito midgut cells by the malaria parasite proceeds by a conserved mechanism and results in death of the invaded midgut cells." Proceedings of the National Academy of Sciences 97(21): 11516-11521.

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VIII. ERKLÄRUNG UND EIGENANTEIL

Hiermit versichere ich, dass ich diese Dissertation selbst verfasst habe und sie – abgesehen von der Beratung durch meine Betreuer – nach Inhalt und Form meine eigene Arbeit darstellt und dass ich keine anderen als die angegebenen Hilfsmittel und Quellen verwendet habe.

Kapitel III

Die Experimente in Kapitel III wurden z.T. mit Hilfe von Corinna Waider durchgeführt, die für Ihre Bachelorarbeit Verhaltensbeobachtungen in der Infektionsphase durchführte. Sie half insbesondere bei der Pflege der Regenwürmer und bei der Infektion.

Kapitel IV

Kapitel IV wurde in Eigenarbeit erstellt.

Kapitel V

Die Sammlung der Regenwürmer für Kapitel V wurde mittels freiwilliger Helfer bewerkstelligt. Dr. Ruxandra Molnar entwickelte mit mir zusammen das Verfahren zur Genotypisierung von *Monocystis* mit Hilfe der M13 Methode, welches ich dann am Max Planck Institut in Tübingen durchführen konnte.