ETH zürich

ETH Library

Positive and negative frequency-dependent parasitism in naturally co-occurring diploid sexual and polyploid asexual Lumbriculus variegatus

Journal Article

Author(s):
Tardent, Nadine; Schlegel, Tamara; Jokela, Jukka ; Hartikainen, Hanna

Publication date:
2025-06

Permanent link:
https://doi.org/https://doi.org/10.3929/ethz-b-000738193

Rights / license:
Creative Commons Attribution 4.0 International

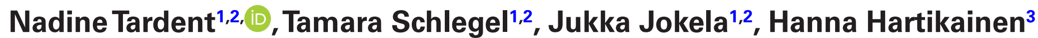
Originally published in:
Journal of Evolutionary Biology 38(6), https://doi.org/10.1093/jeb/voaf046

This page was generated automatically upon download from the ETH Zurich Research Collection.
For more information, please consult the Terms of use.

Journal of Evolutionary Biology, 2025, **38**, 769–777
https://doi.org/10.1093/jeb/voaf046
Advance access publication 24 April 2025
Research Article

JOURNAL OF
Evolutionary Biology
eseb European Society for Evolutionary Biology

Positive and negative frequency-dependent parasitism in naturally co-occurring diploid sexual and polyploid asexual *Lumbriculus variegatus*



Nadine Tardent[1,2], Tamara Schlegel[1,2], Jukka Jokela[1,2], Hanna Hartikainen[3]

[1]Department of Aquatic Ecology, Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf, Switzerland
[2]Institute of Integrative Biology, ETH Zurich, Zurich, Switzerland
[3]School of Life Sciences, University of Nottingham, Nottingham, United Kingdom

Handling editor: Trine Bilde, Associate Editor: Erica Larson

Corresponding author: Nadine Tardent, Swiss Federal Institute of Aquatic Science and Technology (Eawag), Department of Aquatic Ecology, Überlandstrasse 133, 8600 Dübendorf, Switzerland. Email: nadine.tardent@eawag.ch

Downloaded from https://academic.oup.com/jeb/article/38/6/769/8119320 by NADEL user on 19 August 2025

Abstract

Polyploidization is an important evolutionary force. It drives sympatric speciation through reproductive isolation of different cytotypes, and often leads to loss of sexual reproduction in polyploid lineages. Polyploidization and asexuality can change how other species engage in ecological interactions with the polyploid lineage and may change coevolutionary dynamics. Here, we quantified the phenotypic divergence in the freshwater oligochaete worm *Lumbriculus variegatus*, the California blackworm, among its co-occurring sexual diploid (Lineage II) and asexual polyploid (Lineage I) lineages. We further investigated variation in parasite communities and infection prevalence among sympatric and allopatric diploid/polyploid populations. 10 out of 18 populations showed co-existence of both lineages, with 7 populations harbouring only the polyploid lineage. Both worm lineages hosted endoparasitic nematodes, an ectoparasitic rotifer, and one potentially symbiotic gut ciliate. The parasite community similarity and overlapping size range of diploid and polyploid worms points to the ecological similarity of the worm lineages, despite the substantial ploidy and reproductive strategy differentiation. Although parasite prevalence varied independently of worm lineage, the prevalence was associated with the frequency of local cytotypes. Specifically, the rotifer prevalence was highest on the rare local cytotype, and nematode prevalence was highest on the common local cytotype. These results suggest the presence of both positive and negative frequency-dependent parasitism, which may contribute to the co-existence in the *L. variegatus* species complex.

Keywords: Polyploidy, frequency dependent parasitism, Lumbriculus variegatus, facultative sexual, asexual

Introduction

Polyploidization can promote rapid sympatric speciation because it is often associated with reproductive isolation (Mayr, 1996; Otto & Whitton, 2000). Despite the large genomic differences that arise especially during allopolyploidization, the evolution of morphological speciation can take a long time, and cryptic species arise readily (Fišer & Koselj, 2022). Despite being morphologically similar to their diploid counterparts, polyploid lineages can differ substantially in ecologically relevant traits and ecological interactions, thus evolving niche differentiation and significantly contributing to the biological complexity of communities (Levin, 1983; Rothfels & Otto, 2016). A wide range of traits have been shown to vary among conspecific ploidy lineages, including susceptibility to predators, competitive success, parasite load, and parasite-induced mortality (e.g., in *Gammarus* species, Galipaud et al., 2017; Rousset et al., 1996; Thomas et al., 1995; or in *Hyalella* species, Cothran et al., 2013). Many of the traits associated with successful evolutionary transitions to polyploidy confer ecological resilience, and robustness to harsh conditions, mediated by heterosis, gene redundancy, and a change to asexual reproduction (Comai, 2005; Van de Peer et al., 2021).

Polyploidy clearly appears to confer advantages that influence distributions, with polyploid lineages generally showing a broader habitat range than conspecific diploids, both in terms of latitude and altitude (Ramsey & Ramsey, 2014). A potential mechanism for the wider dispersal of polyploids is the association of polyploidization with obligate asexual reproduction. Asexuals have an advantage in that single individuals can establish populations (reproductive assurance). Asexual lineages should have a faster growth rate as they do not need to produce males (cost of males) or engage in searching for mates (Jokela et al. 1997; Maynard Smith, 1971, 1978). Therefore, asexual lineages should have a colonization advantage coupled with a faster growth rate than sexual lineages, driving the adoption of wider habitat ranges (geographical parthenogenesis, Tilquin & Kokko, 2016; Vandel, 1928). It follows that the polyploidization and asexual reproduction often co-occur, making it difficult to separate the relative ecological and evolutionary advantages of each process.

The ability to adapt to coevolving parasites is suggested as a major benefit of sexual reproduction (Bell, 1982; Hamilton, 1980). Polyploidization often leads to parthenogenesis which is traditionally expected to make lineages easier targets for parasite coevolution. However,

Received January 10, 2025; revised March 13, 2025; accepted April 21, 2025
© The Author(s) 2025. Published by Oxford University Press on behalf of the European Society of Evolutionary Biology.
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

polyploidization may also alter the genes coding for immune defences and elicit change in morphological traits. Therefore, polyploidization can have various impacts on traits associated with parasite resistance and susceptibility. However, empirical studies investigating how ploidy affects parasite resistance are relatively scarce, but theoretical expectations are clear. Allelic diversity, increased heterozygosity, and higher expression levels of immune-relevant genes could all improve polyploid immune function (King et al., 2012). Indeed, theoretical models predict newly formed polyploids to have an increased resistance to parasites compared to their diploid progenitors (Oswald & Nuismer, 2007). In a comparison of diploid and putative tetraploid catfish, polyploidy was associated with lower number of parasites and higher diversity in two toll-like receptor genes (Bell et al., 2020), while diploid and polyploid soybean (*Glycine tomentella*) showed similar resistance to a fungal pathogen (Schoen et al. 1992). Increased ploidy likely has a complex effect on immune function in animals and plants with correlations spanning all directions (King et al., 2012; Segraves & Anneberg, 2016). Further, most studies on interactions of ploidy and parasitism rely on laboratory experiments and extrapolation to natural systems may be difficult. Harms et al. (2020) showed that although the polyploid plant *Butomus umbellatus* suffered from larger fungal leaf lesions in experimental assays, infection frequencies in the field were lower compared to diploid conspecifics. It remains crucial to understand the independent effects of asexuality and increased ploidy on parasite resistance. However, it is equally important to examine how asexual polyploids differ in their resistance compared to sexual diploids, as these groups often represent distinct evolutionary strategies found in natural populations.

The goal of this study was to reveal whether parasite communities and prevalences varied among naturally occurring, sympatric diploid, and polyploid lineages. We focused on an aquatic oligochaete worm *Lumbriculus variegatus,* which comprises conspecific lineages of diploid and polyploid worms. The diploid lineage (Lineage II, Gustafsson et al., 2009) undergoes both sexual and asexual reproduction. The asexual form of reproduction is via fission (architomy). The exact mode of sexual reproduction is currently not known, but the worms develop sexual organs during the summer months and form cocoons. Eggs and subsequently embryos develop within these cocoons and several new worms hatch (Drewes & Brinkhurst 1990, pers. obs. N. Tardent). Tweeten and Scollick (2020) have observed simultaneous sperm and egg formation within individuals. Additionally, tracking of labelled sperm DNA indicated that outcrossing is likely to occur (Tweeten & Scollick, 2020). The frequency of sex has historically been described as rare (Cook, 1969), but we have observed one season in which at least 50% of the diploids produced sexual organs (pers. obs. N. Tardent), as have others (Tweeten & Vang, 2011). The polyploid lineage (Lineage I, Gustafsson et al., 2009) only reproduces asexually via fission. Specifically, we developed a rapid molecular tool to identify the two lineages, based on their mitochondrial 16S sequences, and assessed the corresponding variation in worm size, frequency of co-occurrence of the two worm lineages, and the associated parasite communities. Finally, we tested whether parasite prevalence and parasite load were linked to the frequency of ploidy in the studied populations.

Methods

Study system

Lumbriculus variegatus, a freshwater oligochaete widely used in ecotoxicological studies, has a worldwide distribution. It is described as a complex of “separately evolving metapopulations” or a putative cryptic species complex (Gustafsson et al., 2009). Analyses of mitochondrial COI, 16S, and nuclear ITS markers resolved *L. variegatus* into at least three different lineages, two of which are widespread and commonly sympatric. The subtypes are morphologically indistinguishable but differ in ploidy: Lineage II is presumed to be diploid and Lineage I is highly polyploid (Gustafsson et al., 2009). Based on flow cytometry and chromosome spreads the ploidy level of Lineage II is estimated to be between 10N—12N (Tweeten & Morris, 2016). Both worm lineages can undergo architomy and use fission to reproduce asexually. Sexual reproduction however has only been observed in the diploid Lineage I. *Lumbriculus variegatus* hosts various parasites, although the life-cycles of many remain unresolved. They are intermediate hosts to the nematode *Dioctophyme renale* (Mace & Anderson, 1975) and host *Rhabditis lumbriculi* (van Linstow Otto. 1895). Ectoparasitic rotifers of *L. variegatus* include the highly virulent *Drilophaga bucephalus* which infects their hosts by piercing their skin to feed on the host’s body fluids (May, 1989; Riemann & Kieneke, 2007; Vejdovsky, 1882). Additionally, different potentially parasitic mouthless ciliate species (order Astomatida) have been found to inhabit the midgut and hindgut of *L. variegatus* (Falls, 1972) and while the nature of this relationship is not defined, other ciliates of the order Astomatida are known endosymbionts of terrestrial oligochaetes (Obert et al., 2021) or freshwater turbellarians (Rataj & Vďačný, 2018).

Worms (n = 40 /site) were collected from 18 sites across Switzerland (Figure 1, Supplementary Table S1). Sites were selected based on suitability of the habitat, which was easy to determine as search for the worms was not successful only in two of the 20 sites visited. Worms were collected from shallow areas of ponds (maximum knee deep), streams, and lakes by sieving sediment using a kitchen sieve with approximately 1 mm mesh size. Only 19 worms were found in the party-garden pond of location Small E (KBS). Worms were placed in individual 15 mL falcon tubes filled with water from the site and transported in a coolbox with iceblocks to the laboratory. All worms were processed within 48 hours of collection. All worms were placed individually in 6-well plates and photographed with a reference scale in the picture. A small piece was cut off from each worm and saved at −20 °C in 100% ethanol. Half of all individuals per site (n = 20/site), but including all individuals of site Seeheim, were crushed between microscope slides and screened for parasites using a dissection microscope at x10-40 magnification. After examination, the crushed material was transferred into 100% ethanol. Potential parasites were classified into four categories: Nematodes, ectoparasitic rotifers, gut ciliates, and others (Supplementary Table S2).

DNA extraction

DNA was extracted from worm pieces and the preserved tissue samples after crushing using the sbeadex Tissue DNA Purification Kit (LGC, Biosearch Technologies) with the following changes to the manufacturer’s protocol: Lysis buffer TN was replaced by lysis buffer PN, and worm tissue was

Downloaded from https://academic.oup.com/jeb/article/38/6/769/8119320 by NADEL user on 19 August 2025

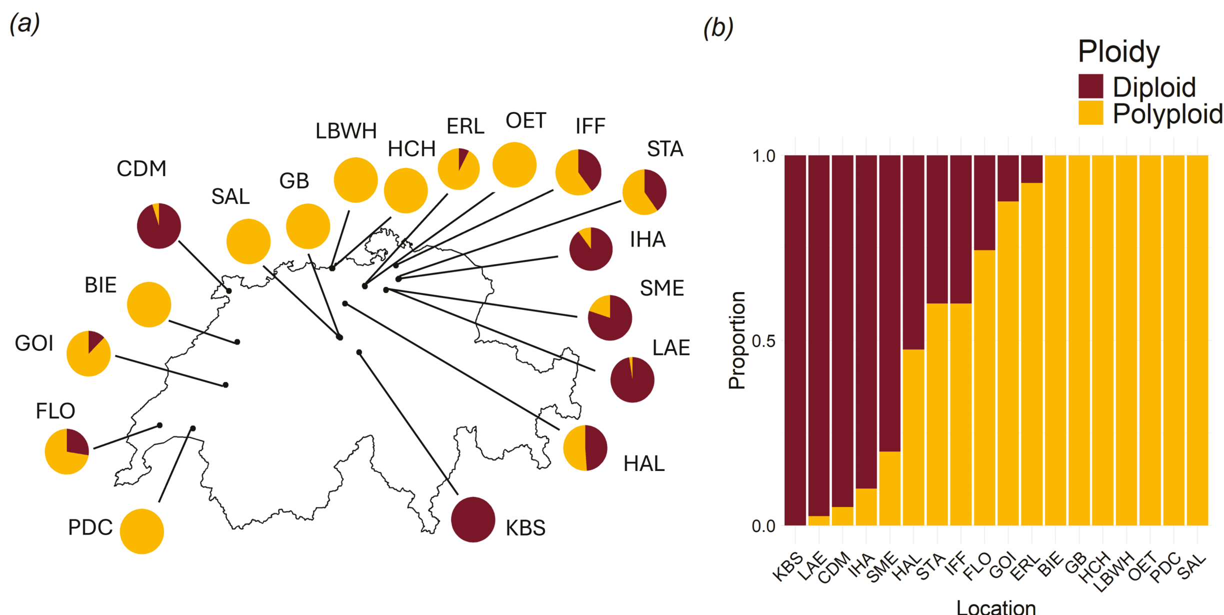


Figure 1: (a) Distribution of *Lumbriculus variegatus* ploidy groups in Switzerland. Polyploid (Lineage I) and diploid (Lineage II) *L. variegatus*. Each pie represents 40 individuals with the exception of sites Seeheim (KBS, $n = 19$), Flogere (FLO, $n = 39$) and Hintere Chalchofe (HCH, $n = 39$). (b) Proportion of lineages II (Diploid) and I (Polyploid) per site, at increasing polyploid Lineage I proportions.

lysed for 2 hr at 55 °C. After lysis, 10 μl of RNase A (10 mg/ml) was added and the mix was incubated at 37 °C for 30 min to remove residual RNA. After the RNase step, the lysate was washed and eluted using a magnetic bead clean-up with the KingFisher Flex Purification Systems (Thermo Scientific) following the sbeadex Tissue DNA Purification Kit instructions. The extracted DNA was eluted in 63 μl elution buffer TN.

Determining worm lineage via 16S subgroup

The diploid and polyploid *L. variegatus* lineages possess a clear difference in the mitochondrial 16S haplotype (Gustafsson et al., 2009), serving as a proxy for the cytotype. To enable the processing of many samples without the need for sequencing, a restriction-digest-based identification of the 16S subgroup was developed. The extracted DNA was amplified using the mitochondrial 16S region primers 16sarL (5′ - CGC CTG TTT ATC AAA AAC AT – 3′) and 16SbrH (5′ - CCG GTY TGA ACT CAG ATC AYG - 3′) modified after Palumbi et al. (1991, modifications highlighted) using a GoTaq G2 Flexi MasterMix (Promega Corporation, Madison WI, USA, Supplementary Table S3) and the following PCR protocol: Initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95°C for 45 seconds, 56°C for 45 seconds and 72 °C for 2 min with a final elongation at 72 °C for 5 min. The expected amplicon size for both lineages was 525 bp. The amplicon was digested using the restriction enzyme KpnI (NEB #R0142S, New England Biolabs Inc., Ipswich, MA, USA) which only cuts the polyploid 16S haplotypes (cutsite 5′-GGTAC^{v}C-3′, Supplementary Figure S1). The PCR fragments were digested according to the manufacturer's recommendations for single digest protocols with 5 μl of PCR product, 1 μl of KpnI enzyme, and NEBuffer 1.1 (Supplementary Table S4). The digested PCR amplicon was visualized using QIAxcel (Qiagen, Hilden, Germany) using a QIAxcel DNA Screening Kit with 5 μl injection volume and 10 s injection time. The 16S haplotype group was assigned based on the observed restriction pattern and assumed to indicate the diploid and polyploid lineages.

Nematode PCR and sanger sequencing

Samples that had at least one nematode were amplified using nematode primers Nem_18S-F(5′- CGC GAA TRG CTC ATT ACA ACA GC -3′) and Nem_18S-R (5′-GGG CGG TAT CTG ATC GCC-3′) which amplify roughly 1kb of the 18S region specific to the phylum Nematoda (Floyd et al., 2005). We used a GoTaq G2 Flexi MasterMix (Promega Corporation, Madison WI, USA) with the same reaction setup as for the 16S fragment (Supplementary Table S3) and the following PCR protocol: Initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 62 °C for 30 s and 72 °C for 1 min with a final elongation at 72 °C for 10 min. The amplicon was visualized using the QIAxcel (Qiagen, Hilden, Germany) using a QIAxcel DNA Screening Kit with 1 μl injection volume and 10 s injection time. Samples with detectable amplicons were then cleaned using a PEG clean (see Supplementary Information section 5). Amplicon concentrations were too low for Sanger sequencing, so the cleaned amplicons were amplified a second time according to the protocol above with 30 cycles, loaded on a 0.8% agarose gel and desired bands were cut out and cleaned up using the MinElute Gel Extraction Kit (Qiagen, Hilden, Germany) according to the MinElute Gel Extraction Kit Protocol using a microcentrifuge. Samples were then prepared for Sanger sequencing and Sanger sequencing was conducted using BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA) at the Genetic Diversity Centre Zurich (see Supplementary section 6). Results were analysed with Geneious Prime 2023.2.1. BLAST searches were done using the nucleotide collection and the Megablast algorithm.

Image analysis

Worm images were analysed using phenopype (Lürig, 2022) following the phenopype gallery project 7 pipeline (https://www.phenopype.org/gallery/) with a reference scale placed in each picture (see Supplementary section 7). In instances

Downloaded from https://academic.oup.com/jeb/article/38/6/769/8119320 by NADEL user on 19 August 2025

where the reference was not properly detected, the reference scale was added manually. A rectangular mask was drawn around the 6-well plate, after which the pipeline automatically detected the worms, skeletonized them, and drew their body outline. In instances where the outline overlapped with a well edge, we carefully removed the wrong pixels manually. The length and area of each worm were extracted in pixels and calculated to mm or mm^2, respectively, according to the mm to pixel ratio recorded by the automatic scale detection.

On average three to four pictures were taken and analysed per worm, and the mean of these measurements was calculated to account for differences due to movement of the worms. Forty worms produced one good photograph for measurements and 27 worms were measured at least ten times, out of which two worms were measured 19 times. Because the worms have different widths along their body, we used area as a proxy for total size.

Statistical analyses

We compared length and area of polyploid and diploid worms in sites where they coexisted and the rare cytotype occurred more than once ($n = 9$ sites). Length and area were log transformed and analysed using generalized linear models with ploidy and location as fixed factors (McCullagh & Nelder, 1983). In these worms, area is a more robust predictor of size than length as worms can extend and subtract even if their body volume does not change. Therefore, we used area as an explanatory variable in all subsequent analyses.

Probability of nematode or rotifer infection was modelled with a generalized linear mixed model (GLMM) with a logit link function and binomial distribution using ploidy and ciliates (categorized into none, few, medium, many) as fixed factors, area as a covariate, and location as a random effect. Infection load was modelled on infected individuals with a GLMM with a log link function and Poisson distribution and ploidy and ciliates as fixed factors, area as a covariate, and location as random effect. The possible difference in the distribution of ciliates (none, few, medium, many) between diploid and polyploid *L. variegatus* was tested using a Chi-square test of independence. Additionally, we tested whether parasite prevalence (nematodes, rotifers) and parasite load were associated with frequency of polyploids per site. For this, we used only locations where both cytotypes coexisted and the rare cytotype occurred more than once ($n = 9$ sites). Probability of parasite infection at locations where both diploid and polyploid worms coexisted was assessed with a general linear model (GLM) using a logit link function and binomial distribution. In this model, ploidy, frequency of polyploids, interaction of ploidy and frequency of polyploids, abundance class of ciliates (none, few, medium, many) were used as fixed effects, and worm area as a covariate. The frequency of polyploids at the location was logit transformed to linearize the predictor. Parasite load at locations where both cytotypes coexisted was tested using infected worms only and required a GLM with a negative binomial distribution and log link function to account for overdispersion. The fixed effects were the same as in the model predicting probability of infection. Fixed effects for all models were tested with a type III Anova. All graphs were produced using the library ggplot2 (v. 3.5.1, Wickham, 2016). We conducted all analyses with R version 4.4.1 (R Core Team, 2024) using R Studio (Posit Team, 2023). We used the libraries dplyr (Wickham et al., 2023), readxl (Wickham & Bryan, 2023), tidyr (Wickham et al., 2024), car (Fox & Weisberg, 2019), lme4 (Bates et al., 2015), and glmmTMB (Brooks et al., 2017).

Results

A total of 697 worms from 18 locations were included in the analysis, with 235 and 462 worms assigned to Lineage II and the polyploid Lineage I, respectively (Gustafsson et al., 2009). Two individuals (from locations Flogere, FLO, and Hintere Chalchofe, HCH) could not be assigned to lineages because PCR of the 16S rRNA gene failed and were excluded from subsequent analyses.

In seven locations only polyploid individuals were found, while in one location—Seeheim (KBS), where only 19 worms instead of the intended 40 were found—only individuals of the diploid Lineage II were present. The two lineages coexisted in 10 of the 18 populations, with varying proportions (Figure 1).

Morphometric analysis of the worms in coexisting sites revealed that polyploids had a larger area ($F_{1,339} = 4.66$, $p = 0.03$, Figure 2) but had the same length as the diploid cytotypes $F_{1,339} = 2.55$, $p = 0.11$, Supplementary Figure S2). Length and area differed between sites (site effect for area: $F_{8,339} = 12.53$, $p < 0.001$, site effect for length: $F_{8,339} = 9.45$, $p < 0.001$).

We found three common groups of organisms associated with *L. variegatus*: Ectoparasitic rotifers, gut ciliates, and nematodes. All three groups were found in both diploid and polyploid *L. variegatus*. Details of the types of parasites recorded are provided in Supplementary Materials Section 1 and where possible taxonomic affiliations were determined by sequencing. For nematodes, the 18S PCR produced three samples with sufficient amplicons. They were all from polyploid samples from locations Erliweiher (ERL, GenBank Acc. No. PV264890), Hintere Chalchofe (HCH, GenBank Acc. No. PV264889) and Oetwil (OET, GenBank Acc. No. PV264888). Two out of the three nematode samples had 100% pairwise identity with each other, while one sample, the sample from Oetwil, only had a pairwise identity of 81% to the other two. The highest pairwise identity match for the two identical samples in the BLAST query was *Paracapillaria najae* (84.3%) and the highest pairwise identity match for the sample from Oetwil was *Paracapillaria siamensis* (92.7%). The three sequenced nematodes had a pairwise identity of 63.9% (two identical sequences) and 69.9% (Oetwil sequence) to

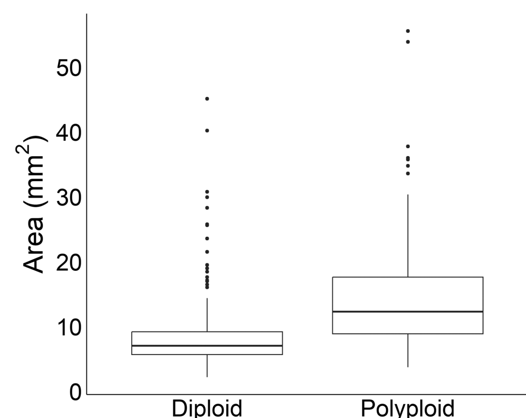


Figure 2: Area (in mm^2) of diploid ($n = 175$) and polyploid ($n = 182$) *Lumbriculus variegatus* in coexisting sites ($n = 9$).

Downloaded from https://academic.oup.com/jeb/article/38/6/769/8119320 by NADEL user on 19 August 2025

Dioctophyme renale 18S and did not produce a contig for *Rhabditis sp*.

Although we were not able to distinguish between different types of ciliates in this study, their overall prevalence was similar in diploids and polyploids (55% of diploid and 49% of polyploid worms, $\chi^2_3 = 5.36$, $p = 0.15$). As ciliates were highly prevalent, and similarly present in both lineages, the presence of ciliates was included as a factor in subsequent analyses on nematode and rotifer prevalence and infection intensity.

We found nematodes in 16.4% of all samples, specifically, 25% of all diploids and 11.7% of all polyploids had at least one nematode. Nematode prevalence was not influenced by ploidy ($\chi^2_1 = 1.67$, $p = 0.20$), ciliates ($\chi^2_3 = 1.49$, $p = 0.68$), or host size ($\chi^2_1 = 1.67$, $p = 0.20$). When nematodes were present, the larger worms had a significantly higher nematode load ($\chi^2_1 = 8.61$ $p = 0.003$) but this did not vary between ploidy lineages ($\chi^2_1 = 0.93$, $p = 0.33$) or among worms with different loads of ciliates ($\chi^2_3 = 6.51$, $p = 0.09$). To investigate potential trade-offs in parasite prevalence and load between diploid and polyploid lineages, the data were limited to the sites where the two ploidy types coexisted and the rarer lineage had more than one individual ($n = 9$). In the shared habitats, the nematode prevalence was higher on the common cytotype (significant interaction of ploidy and proportion of polyploids, $\chi^2_1 = 10.38$, $p = 0.001$, Figure 3). Higher nematode loads in coexisting sites were again marginally biased towards larger worms (area; $\chi^2_1 = 3.26$, $p = 0.07$) but did not differ in any other tested factors (ploidy ($\chi^2_1 = 0.14$, $p = 0.7$), proportion of polyploids ($\chi^2_1 = 1.79$, $p = 0.18$) or ciliates ($\chi^2_1 = 0.72$, $p = 0.86$)).

Rotifers were observed in 14% of all worms. Specifically, 10% of all diploids and 15.6 % of all polyploids had at least one ectoparasitic rotifer attached. The prevalence of rotifers did not differ between ploidy ($\chi^2_1 = 0.22$ $p = 0.64$), size ($\chi^2_1 = 2.27$, $p = 0.13$) or ciliate load ($\chi^2_3 = 1.35$, $p = 0.72$). Infection load, however, differed between ploidy ($\chi^2_1 = 33.05$, $p < 0.001$), ciliates ($\chi^2_3 = 12.40$, $p = 0.006$) and worm size ($\chi^2_1 = 6.39$, $p = 0.01$). Polyploid *L. variegatus* had a higher infection load compared to diploids, and worms carrying either medium or many ciliates had a lower infection load and larger worms hosted more rotifers. While diploid worms harboured up to three rotifers per worm, polyploid specimens were often found to be infected by more than 5 and as many as 26 per individual (Figure 3). In sites where the two ploidy types coexisted, diploid worms had a high rotifer prevalence when diploids were rare and polyploids had a high rotifer prevalence when polyploids were rare (significant interaction of ploidy and proportion of polyploids, $\chi^2_1 = 12.25$ $p < 0.001$, Figure 3). Note that this pattern is opposite to nematodes, where the common cytotype was disproportionately infected. In sites where both cytotypes coexisted, rotifer load differed significantly between ploidy ($\chi^2_1 = 8.09$, $p = 0.005$) with

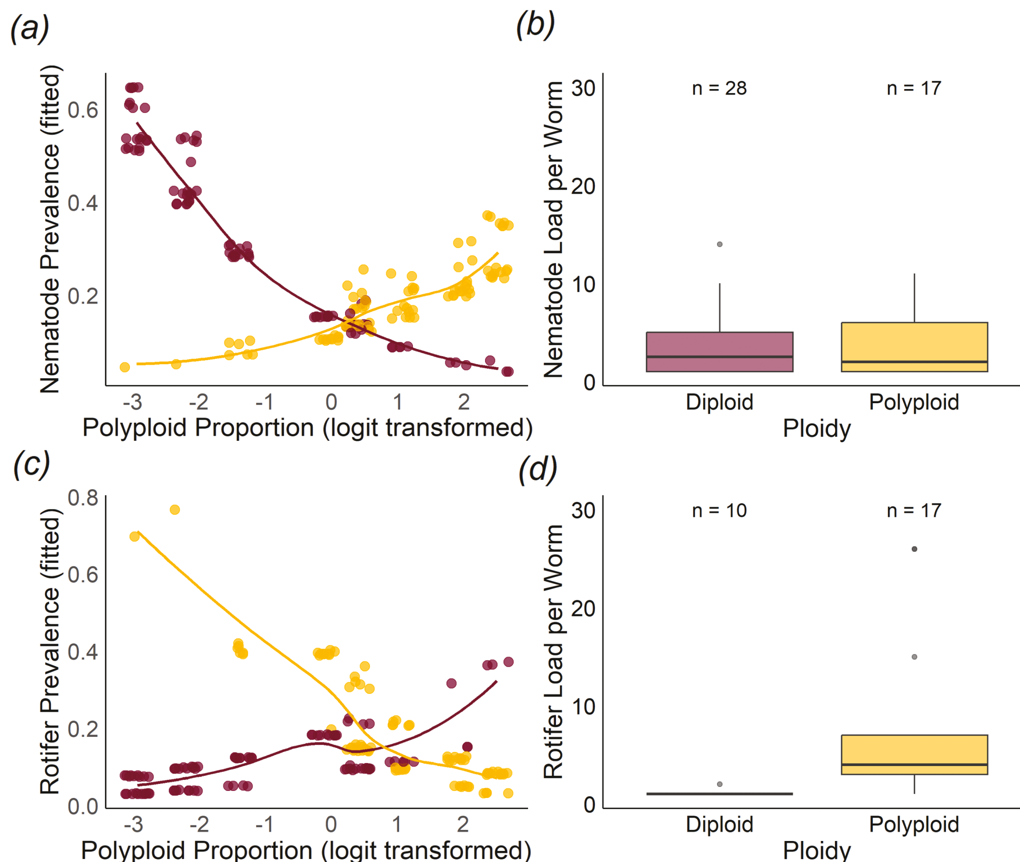


Figure 3: Prevalence and parasite load of diploid (Lineage II) and polyploid (Lineage I) *Lumbriculus variegatus* cytotypes in coexisting sites ($n = 9$). (a) Fitted nematode prevalence (GLM, logit link, binomial family) versus logit transformed polyploid proportion shows that the more common cytotype in a location is predominantly infected with nematodes. The fitted curve is a LOESS smoother. (b) Nematode load per worm in coexisting sites does not differ between ploidy. (c) Fitted rotifer prevalence (GLM, logit link, binomial family) versus logit transformed polyploid proportion shows that the rare cytotype is disproportionately more infected than the common cytotype. The fitted curve is a LOESS smoother. (d) Rotifer load in coexisting sites is higher on polyploid than on diploid worms.

Downloaded from https://academic.oup.com/jeb/article/38/6/769/8119320 by NADEL user on 19 August 2025

higher rotifer load for polyploids than diploids. Finally, the rotifer load did not differ by ciliates ($\chi^2_3 = 4.53$, $p = 0.21$) or size ($\chi^2_1 = 1.30$, $p = 0.25$).

Discussion

Theory predicts polyploids to be more widespread than their diploid counterparts (Ramsey & Ramsey, 2014; Tilquin & Kokko, 2016) and suggests that they could show enhanced parasite resistance owing to an increased number of genes involved in immune defences (King et al., 2012; Oswald & Nuismer, 2007). Here, we show that in *Lumbriculus variegatus*, obligately asexual polyploids are more prevalent and more widespread, but also note that coexistence of the polyploid and optionally sexually reproducing diploid worm lineage is very common. We also show that parasite resistance cannot be explained by ploidy alone, but rather by local ploidy frequency.

Despite the likely complete reproductive isolation between the two lineages, the worms show little morphological, niche or parasite community differentiation. Further, the two worm lineages share three groups of associated organisms—an ectoparasitic rotifer, gut ciliates and nematodes. Nematodes had a higher prevalence on the locally common cytotype and rotifers had a higher prevalence on the locally rare cytotype.

Polyploid *L. variegatus* were significantly more widespread than their diploid counterparts. Two thirds of the collected specimens were polyploid and in seven of the 18 locations they were the only recorded cytotype. While previous studies have shown that polyploids tend to inhabit higher latitudes than diploids (David, 2022), it remains unclear whether they have larger habitat ranges (Tilquin & Kokko, 2016). However, it is well documented that polyploids are more robust to both abiotic and biotic stressors (Chao et al., 2013; Hannweg et al., 2016; Švara et al., 2021; Van de Peer et al., 2021), which may explain their wider distribution among small waterbodies. Polyploidy may come with other related benefits, such as more efficient asexual reproduction via fission due to lack of investment in sexual reproduction, which is associated with a generally broader habitat (geographical parthenogenesis, (Vandel, 1928)) and the ability to regularly colonize newly available habitats (Hörandl, 2009).

The distribution of the diploid lineage or the sympatric sites did not clearly follow a spatially clustered pattern, nor was there an attributable link to habitat permanency, suggesting no clear niche differentiation between the worm lineages. The habitats which harboured *L. variegatus* all had different properties; some had a visible water inlet and outlet, while others were fed by groundwater or, from what we could observe, precipitation only. Permanency of sampling sites likely varied, e.g., we observed several locations to drastically respond to drought (Flogere, FLO), almost dry out (Im Hau, IHA), or completely vanish (Small E, SME). Notably, both Im Hau and Small E harboured a high number of potentially sexual diploids. *L. variegatus* sexual reproduction results in a production of a resistant cocoon, which may be beneficial during seasonal drought. Many aquatic organisms use similar strategies, such as *Daphnia sp.*, where sexually produced resting stages (ephippia) are deposited in the sediment when conditions are deteriorating (Ebert 2005) or at high population densities (Gerber et al., 2018). Due to the low sample size and variation in permanency, it remains unclear whether habitat stochasticity has an influence on the distribution of the different *L. variegatus* lineages, and whether the two lineages exhibit differences in their metapopulation dynamics.

Although considered morphologically very similar, the diploid and polyploid *L. variegatus* were found to differ in size. Generally, polyploids had a bigger surface area, but they were not longer. While this holds true for the mean of the two lineages overall, it is still difficult to distinguish the two cytotypes using this criterion due to the overlap in size distributions and the observed strong site-specific variation in size. Mature sexual diploid *L. variegatus* were of similar size to the biggest polyploid specimens, which might suggest that the observed size distributions of the two cytotypes may differ depending on the sampling season. Combining these findings with the genetic analyses (COI, 16S, and ITS, Gustafsson et al., 2009) the two cytotypes appear to be morphologically cryptic species, with ecologically similar requirements.

The symbiont and parasite communities of the two worm lineages were similar, with the frequently encountered groups being nematodes, rotifers, and ciliates. Very little is known regarding the parasites of *L. variegatus*, and identification to lower taxonomic groupings was mostly not possible. Based on literature, we expected to find *Rhabiditis lumbriculi* or *Dioctophyme renale* nematodes (Mace & Anderson, 1975; v. Linstow Otto, 1895), but the closest sequencing match to the collected nematodes were *Paracapillaria sp.*, (Family Capillariidae), which are parasites of mammals and may use oligochaetes as paratenic intermediate hosts (Moravec et al., 1987). Pairwise identity between our samples *Paracapillaria* was only between 84% and 93% which indicates that our collected nematodes could be from a different genus. The prevalence of nematodes was higher among the common cytotype within populations, possibly supporting a frequency-dependent infection process. Additionally, the nematode load increased with host size. This observation aligns with the expected low virulence in paratenic hosts, since bigger worms may have a longer time to accumulate infections. We cannot exclude the possibility that polyploids and diploids are infected by different nematode species, but the positive frequency-dependent parasite distribution between the nematodes and the most common cytotype does not support this.

Previous studies have documented the presence of an ectoparasitic rotifer on *L. variegatus*, *Drilophaga bucephalus* Vejdovsky 1882 (May, 1989; Riemann & Kieneke, 2007; Vejdovsky, 1882) and ectoparasitic *Cephalodella parasitica* on aquatic annelids (May, 1989). We excluded *C. parasitica* as a potential candidate based on morphology and assume the rotifers we encountered is *D. bucephalus*. *Lumbriculus variegatus* has been suggested to act as the main host for *D. bucephalus*, but some records also originate from *Rhynchelmis sp.* and *Nais elinguis* (Koste, 1972). *Drilophaga bucephalus* attaches to the integument of the worm host and pierces the skin to feed (May, 1989), posing significant virulence. In our stock cultures the rotifers can have a substantial negative impact on worms (pers. obs. N. Tardent) and maintaining cultures of the rotifer without their worm hosts has not been successful (pers. comm. D. Fontaneto). Rotifer prevalence was negatively frequency dependent, meaning that the two cytotypes had lower rotifer prevalences when they were common and higher prevalences when they were rare. Such a pattern could arise from various processes. For example, the parasite could adapt to the locally common cytotype with an increased virulence and incurring death of infected hosts, thus reducing the prevalence in the common host and

Downloaded from https://academic.oup.com/jeb/article/38/6/769/8119320 by NADEL user on 19 August 2025

eventually reducing its local frequency. Such a mechanism should be observed over several years, ideally with frequent sampling of both cytotypes and their parasites. Alternatively, the locally common cytotype could have evolved a higher resistance against infections while the rare cytotype cannot evolve a similar resistance. This could occur if the genetic diversity in the locally rare cytotype is low. In a study of a trematode infection of sexual and asexual *Poeciliopsis monachal* fish higher prevalences of the parasite on the rare sexual population could be attributed to inbreeding depression (Lively et al., 1990). In this system, low genetic diversity in the diploids could arise through inbreeding depression or a shift to asexual reproduction via fission and in the polyploids through low clonal diversity. Genetic data of the both cytotypes across all study sites could help support or refute this hypothesis.

While both diploid and polyploid *L. variegatus* had overall similar rotifer prevalences, rotifer infection intensity on polyploid worms was significantly higher. The higher infection intensity on polyploids could indicate that the polyploid *L. variegatus* are less resistant to rotifer attack, although there is no direct evidence for this currently. Alternatively, it could indicate that polyploid *L. variegatus* can tolerate higher parasite loads, which would give them an advantage in locations where they coexist with diploid worms. Given the observed high virulence of the rotifer, they may have the potential to drive host-parasite coevolution in this system and promote co-existence of the two worm lineages. Parasites promoting coexistence of sexuals and asexuals have been proposed in studies on *Potamopyrgus antipodarum* and its coevolving parasite *Atriophallophorus winterbournii* (Gibson et al., 2018; Jokela et al., 2009). It is difficult to disentangle the relative contributions of resistance and virulence in *L. variegatus—D. bucephalus* system without further data; however, the rotifers do appear to impose a significant cost to their hosts, and show markedly different prevalence patterns to the nematodes with assumed low virulence due to paratenic infection strategy. Laboratory experiments and long-term study of habitats with diploid sexual and polyploid asexual *L. variegatus* infected by *D. bucephalus* will help elucidate how ploidy and reproductive mode influence host parasite dynamics in this natural system.

Half of all examined worms carried gut ciliates, suggesting a prevalent and frequent association. The few documented cases of ciliate detection from *L. variegatus* report at least five different gut ciliates: *Ptychostomum lumbriculi* Heidenreich and *Mesnilella clavata* Leidy, *Ptychostomum chattoni* Rossolimo, *Mesnilella trispiculata* Kejensky and *Hoplitophrya secans* Stein (Falls, 1972). It is unclear whether the symbiotic relationship with *L. variegatus* is parasitic or commensal, as subsequent studies lack any trait information (Heidenreich, 1935; Rossolimo & Perzewa, 1929; Studitsky, 1932). In other systems, astome ciliates have well-established obligate, mostly commensal relationships with lumbricid earthworms (Obert et al., 2021) or aquatic turbellarians (Rataj & Vďačný, 2018).

Interestingly, rotifer infection intensity was negatively correlated with ciliate abundance. Worms with low-intensity rotifer infection generally had medium or many ciliates in their guts, suggesting that the digestive system of a worm under high rotifer attack is no longer able to support strong commensal ciliate populations. Alternatively, the ciliates may play a more direct role in the worm's immune defence. For example, endosymbiotic bacterial *Spiroplasma sp.* affords protection against nematodes in *Drosophila melanogaster* (Eleftherianos et al., 2018) and the endosymbiotic bacterium *Hamiltonella defensa* protects pea aphids against the parasitoid wasp *Aphidius ervi* (Oliver et al., 2005). Further experiments are necessary to determine whether this relationship in *L. variegatus* is causal or not.

Overall, our study shows that even though *Lumbriculus variegatus* consists of two genetically, and to a certain degree, morphologically distinct groups, they are ecologically very similar. This is evident in the frequently observed coexistence in nature and their similarities in associated parasite, commensal, or potentially symbiont communities. The broader implication of the ecological similarity of the two worm lineages is that *L. variegatus* may provide a well-suited model system for assessing the strength of intra- and interspecies competition (Fišer & Koselj, 2022), invasibility (Siepielski & McPeek, 2010) and the conditions under which ecologically stable coexistence arises (Chesson, 2000). The positive and negative frequency-dependent infections of nematodes and rotifers are striking. Future work on this system should focus on the genetic diversity of both cytotypes, especially in the context of being rare versus being common. Additionally, long-term monitoring of *L. variegatus* host parasite populations could show whether our findings are snapshots of complex population dynamics or whether local parasite prevalences are stable.

Supplementary material

Supplementary material is available at *Journal of Evolutionary Biology* online.

Data availability

The data underlying this article are available in the Dryad Digital Repository, at https://doi.org/10.5061/dryad.18931zd76. Sequence data are available on GenBank under Accession Numbers PV264888, PV264889, PV264890.

Author contributions

Nadine Tardent (Conceptualization [Equal], Data curation [Lead], Formal analysis [Lead], Investigation [Lead], Methodology [Equal], Writing - original draft [Lead], Writing - review & editing [Equal]), Tamara Schlegel (Investigation [Supporting], Methodology [Equal], Writing - review & editing [Supporting]), Jukka Jokela (Conceptualization [Equal], Formal analysis [Supporting], Funding acquisition [Equal], Supervision [Supporting], Writing - original draft [Supporting], Writing - review & editing [Equal]), and Hanna Hartikainen (Conceptualization [Equal], Formal analysis [Supporting], Funding acquisition [Equal], Supervision [Lead], Writing - original draft [Supporting], Writing - review & editing [Equal])

Acknowledgments

We thank Bettina Dubach, Rosina Beer, Andrin Krähenbühl, Nora Weissert and Jana Jucker for their help in the field collections and sample processing. Data produced and analyzed in this paper were generated in collaboration with the Genetic Diversity Centre (GDC), ETH Zurich. We also thank

Downloaded from https://academic.oup.com/jeb/article/38/6/769/8119320 by NADEL user on 19 August 2025

two anonymous reviewers for their comments on the manuscript.

Funding

This study was supported by Swiss National Science foundation (#310030_207589 to JJ and #310031_185323 to HH).

Conflicts of interest

We have no conflict of interest to declare.

References

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using **lme4**. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Bell, E. A., Cable, J., Oliveira, C., … Taylor, M. I. (2020). Help or hindrance? The evolutionary impact of whole-genome duplication on immunogenetic diversity and parasite load. *Ecology and Evolution*, *10*(24), 13949–13956. https://doi.org/10.1002/ece3.6987

Bell, G. (1982). *The Masterpiece of Nature: The Evolution and Genetics of Sexuality*. Croom Helm Ltd, London & Canberra.

Brooks, M. E., Kristensen, K., Benthem, K. J., & … Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, *9*(2), 378. https://doi.org/10.32614/RJ-2017-066

Chao, D. -Y., Dilkes, B., Luo, H., … Salt, D. E. (2013). Polyploids exhibit higher potassium uptake and salinity tolerance in *Arabidopsis*. *Science*, *341*(6146), 658–659. https://doi.org/10.1126/science.1240561

Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics*, *31*(1), 343–366. https://doi.org/10.1146/annurev.ecolsys.31.1.343

Comai, L. (2005). The advantages and disadvantages of being polyploid. *Nature Reviews Genetics*, *6*(11), 836–846. https://doi.org/10.1038/nrg1711

Cook, D. G. (1969). Observations on the life history and ecology of some Lumbriculidae (Annelida, Oligochaeta). *Hydrobiologia*, *34*(3-4), 561–574. https://doi.org/10.1007/bf00045410

Cothran, R. D., Henderson, K. A., Schmidenberg, D., & Relyea, R. A. (2013). Phenotypically similar but ecologically distinct: Differences in competitive ability and predation risk among amphipods. *Oikos*, *122*(10), 1429–1440. https://doi.org/10.1111/j.1600-0706.2013.00294.x

David, K. T. (2022). Global gradients in the distribution of animal polyploids. *Proceedings of the National Academy of Sciences of the United States of America*, *119*(48), e2214070119. https://doi.org/10.1073/pnas.2214070119

Drewes, C. D., & Brinkhurst, R. O. (1990). Giant nerve fibers and rapid escape reflexes in newly hatched aquatic oligochaetes, Lumbriculus variegatus (Family Lumbriculidae). *Invertebrate Reproduction & Development*, *17*(2), 91–95. https://doi.org/10.1080/07924259.1990.9672095

Ebert, D. (2005). Ecology, Epidemiology, and Evolution of Parasitism in Daphnia. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information. https://www.ncbi.nlm.nih.gov/books/NBK2036/

Eleftherianos, I., Yadav, S., Kenney, E., … Patrnogic, J. (2018). Role of endosymbionts in insect–parasitic nematode interactions. *Trends in Parasitology*, *34*(5), 430–444. https://doi.org/10.1016/j.pt.2017.10.004

Falls, E. Q. (1972). *A survey of fresh-water oligochaeta and their commensal ciliates from the Richmond, Virginia area* [Master Thesis]. http://scholarship.richmond.edu/masters-theses

Fišer, C., & Koselj, K. (2022). Coexisting cryptic species as a model system in integrative taxonomy. In Monro, A. K., & S. J. Mayo (Eds.), *Cryptic Species: Morphological Stasis, Circumscription, and Hidden Diversity*. Systematics Association Special Volume Series (pp. 169–196). Cambridge University Press. https://doi.org/10.1017/9781009070553.007

Floyd, R. M., Rogers, A. D., Lambshead, P. J. D., & Smith, C. R. (2005). Nematode-specific PCR primers for the 18S small subunit rRNA gene. *Molecular Ecology Notes*, *5*(3), 611–612. https://doi.org/10.1111/j.1471-8286.2005.01009.x

Fox, J., & Weisberg, S. (2019). *An R Companion to Applied Regression* (Third). Sage. https://www.john-fox.ca/Companion

Galipaud, M., Bollache, L., & Lagrue, C. (2017). Variations in infection levels and parasite-induced mortality among sympatric cryptic lineages of native amphipods and a congeneric invasive species: Are native hosts always losing? *International Journal for Parasitology: Parasites and Wildlife*, *6*(3), 439–447. https://doi.org/10.1016/j.ijppaw.2017.04.005

Gerber, N., Kokko, H., Ebert, D., & Booksmythe, I. (2018). Daphnia invest in sexual reproduction when its relative costs are reduced. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1871), 20172176. https://doi.org/10.1098/rspb.2017.2176

Gibson, A. K., Delph, L. F., Vergara, D., & Lively, C. M. (2018). Periodic, parasite-mediated selection for and against sex. *The American Naturalist*, *192*(5), 537–551. https://doi.org/10.1086/699829

Gustafsson, D. R., Price, D. A., & Erséus, C. (2009). Genetic variation in the popular lab worm Lumbriculus variegatus (Annelida: Clitellata: Lumbriculidae) reveals cryptic speciation. *Molecular Phylogenetics and Evolution*, *51*(2), 182–189. https://doi.org/10.1016/j.ympev.2008.12.016

Hamilton, W. D. (1980). Sex versus non-sex versus parasite. *Oikos*, *35*(2), 282–290. https://doi.org/10.2307/3544435

Hannweg, K., Steyn, W., & Bertling, I. (2016). In vitro-induced tetraploids of Plectranthus esculentus are nematode-tolerant and have enhanced nutritional value. *Euphytica*, *207*(2), 343–351. https://doi.org/10.1007/s10681-015-1547-4

Harms, N., Shearer, J., Cronin, J. T., & Gaskin, J. F. (2020). Geographic and genetic variation in susceptibility of *Butomus umbellatus* to foliar fungal pathogens. *Biological Invasions*, *22*(2), 535–548. https://doi.org/10.1007/s10530-019-02109-3

Heidenreich, E. (1935). *Ptychostomum lumbriculi* n. sp. *Archiv Für Protistenkunde*, *85*, 303–305.

Hörandl, E. (2009). Geographical parthenogenesis: opportunities for asexuality. In I. Schön, K. Martens, & P. Dijk (Eds.), *Lost sex: the evolutionary biology of parthenogenesis* (pp. 161–186). Springer Netherlands. https://doi.org/10.1007/978-90-481-2770-2_8

Jokela, J., Dybdahl, M. F., & Lively, C. M. (2009). The maintenance of sex, clonal dynamics, and host-parasite coevolution in a mixed population of sexual and asexual snails. *American Naturalist*, *174*(Suppl 1), S43–S53. https://doi.org/10.1086/599080

Jokela, J., Lively, C. M., Dybdahl, M. F., & Fox, J. A. (1997). Evidence for a cost of sex in the freshwater snail *Potamopyrgus antipodarum*. *Ecology*, *78*(2), 452–460. https://doi.org/10.2307/2266021

King, K. C., Seppälä, O., & Neiman, M. (2012). Is more better? Polyploidy and parasite resistance. *Biology Letters*, *8*(4), 598–600. https://doi.org/10.1098/rsbl.2011.1152

Koste, W. (1972). Über zwei seltene parasitische Rotatorienarten *Drilophaga bucephalus* VEJDOVSYK und *Proales gigantea* (GLASCOTT). *Osnabrücher Naturwissenschaftliche Mitteilungen*, *1*, 149–158.

Levin, D. A. (1983). Polyploidy and novelty in flowering plants. *The American Naturalist*, *122*(1), 1–25. https://doi.org/10.1086/284115

Lively, C. M., Craddock, C., & Vrijenhoek, R. C. (1990). Red Queen hypothesis supported by parasitism in sexual and clonal fish. *Nature*, *344*(6269), 864–866. https://doi.org/10.1038/344864a0

Lürig, M. D. (2022). Phenopype: a phenotyping pipeline for Python. *Methods in Ecology and Evolution*, *13*(3), 569–576. https://doi.org/10.1111/2041-210x.13771

Mace, T. F., & Anderson, R. C. (1975). Development of the giant kidney worm, *Dioctophyma renale* (Goeze, 1782)

Downloaded from https://academic.oup.com/jeb/article/38/6/769/8119320 by NADEL user on 19 August 2025

(Nematoda: Dioctophymatoidea). *Canadian Journal of Zoology*, *53*(11), 1552–1568. https://doi.org/10.1139/z75-190

May, L. (1989). Epizoic and parasitic rotifers. *Hydrobiologia*, *186-187*(1), 59–67. https://doi.org/10.1007/bf00048897

Maynard Smith, J. (1971). The origin and maintenance of sex. In G.C. Williams (Ed.), *Group selection, Aldine Atherton (1971)* (pp. 163–175). Taylor and Francis. https://doi.org/9780203790427

Maynard Smith, J. (1978). *The Evolution of Sex*. Cambridge University Press. https://doi.org/10.1017/S0016672300018693

Mayr, E. (1996). What is a species, and what is not? *Philosophy of Science*, *63*(2), 262–277. https://doi.org/10.1086/289912

McCullagh, P., & Nelder, J. (1983). *Generalized linear models*. Chapman and Hall.

Moravec, F., Prokopič, J., & Shlikas, A. V. (1987). The biology of nematodes of the family Capillariidae Neveu-Lemaire, 1936. *Folia Parasitologica*, *34*(1), 39–56.

Obert, T., Rurik, I., & Vd'ačný, P. (2021). Diversity and eco-evolutionary associations of endosymbiotic astome ciliates with their lumbricid earthworm hosts. *Frontiers in Microbiology*, *12*, 12. https://doi.org/10.3389/fmicb.2021.689987

Oliver, K. M., Moran, N. A., & Hunter, M. S. (2005). Variation in resistance to parasitism in aphids is due to symbionts not host genotype. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(36), 12795–12800. https://doi.org/10.1073/pnas.0506131102

Oswald, B. P., & Nuismer, S. L. (2007). Neopolyploidy and pathogen resistance. *Proceedings Biological Sciences*, *274*(1624), 2393–2397. https://doi.org/10.1098/rspb.2007.0692

Otto, S. P., & Whitton, J. (2000). Polyploid incidence and evolution. *Annual Review of Genetics*, *34*, 401–437. https://doi.org/10.1146/annurev.genet.34.1.401

Palumbi, S. R., Martin, A., Romano, S., McMillan W.O., Stice, L., Grabowski, G. (2002). *The Simple Fool's Guide to PCR, Version 2.0*. Department of Zoology and Kewalo Marine Laboratory.

Posit Team. (2023). *RStudio: Integrated development environment for R*. Posit Software, PBC.

R Core Team. (2024). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Ramsey, J., & Ramsey, T. S. (2014). Ecological studies of polyploidy in the 100 years following its discovery. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *369*(1648), 20130352. https://doi.org/10.1098/rstb.2013.0352

Rataj, M., & Vďačný, P. (2018). Dawn of astome ciliates in light of morphology and time-calibrated phylogeny of *Haptophrya planariarum*, an obligate endosymbiont of freshwater turbellarians. *European Journal of Protistology*, *64*, 54–71. https://doi.org/10.1016/j.ejop.2018.03.004

Riemann, O., & Kieneke, A. (2007). *Drilophaga bucephalus* - Ein seltenes ektoparasitisches Rädertier. *Mikrokosmos*, *96*(5), 263–267.

Rossolimo, L. L., & Perzewa, T. A. (1929). Zur Kenntnis einiger astomen infusorien: Studien an skelettbildung. *Archiv Für Protistenkunde*, *68*(67), 237–252.

Rothfels, C. J., & Otto, S. P. (2016). Polyploid speciation. In Kliman, R. M. (Ed.), *Encyclopedia of evolutionary biology* (pp. 317–326). Academic Press. https://doi.org/10.1016/B978-0-12-800049-6.00073-1

Rousset, F., Thomas, F., De Meeus, T., & Renaud, F. (1996). Inference of parasite-induced host mortality from distributions of parasite loads. *Ecology*, *77*(7), 2203–2211. https://doi.org/10.2307/2265713

Schoen, D. J., Burdon, J. J., & Brown, A. H. D. (1992). Resistance of Glycine tomentella to soybean leaf rust Phakopsora pachyrhizi in relation to ploidy level and geographic distribution. *Theoretical and Applied Genetics*, *83–83*(6-7), 827–832. https://doi.org/10.1007/BF00226704

Segraves, K. A., & Anneberg, T. J. (2016). Species interactions and plant polyploidy. *American Journal of Botany*, *103*(7), 1326–1335. https://doi.org/10.3732/ajb.1500529

Siepielski, A. M., & McPeek, M. A. (2010). On the evidence for species coexistence: a critique of the coexistence program. *Ecology*, *91*(11), 3153–3164. https://doi.org/10.1890/10-0154.1

Studitsky, A. N. (1932). Über die morphologie, cytologie und systematik von ptychostomum chattoni rossolimo. *Archiv Für Protistenkunde*, *76*, 188–216.

Švara, A., Ilnikar, K., Carpentier, S., … Keulemans, W. (2021). Polyploidy affects the development of Venturia inaequalis in scab-resistant and -susceptible apple cultivars. *Scientia Horticulturae*, *290*(2), 110436. https://doi.org/10.1016/j.scienta.2021.110436

Thomas, F., Renaud, F., Rousset, F., … De Meeus, T. (1995). Differential mortality of two closely related host species induced by one parasite. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *260*(1359), 349–352. https://doi.org/10.1098/rspb.1995.0103

Tilquin, A., & Kokko, H. (2016). What does the geography of parthenogenesis teach us about sex? *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1706), 20150538. https://doi.org/10.1098/rstb.2015.0538

Tweeten, K. A., & Morris, S. J. (2016). Flow cytometry analysis of DNA ploidy levels and protein profiles distinguish between populations of Lumbriculus (Annelida: Clitellata). *Invertebrate Biology*, *135*(4), 385–399. https://doi.org/10.1111/ivb.12150

Tweeten, K. A., & Scollick, J. (2020). Tracking of labeled sperm suggests that diploid populations of sexually reproducing lumbriculus cross fertilize. In Meeting Abstract. Annual Meeting of the Society of Integrative and Comparative Biology.

Tweeten, K. A., & Vang, C. (2011). Observations on sexual reproduction in *Lumbriculus variegatus*. In Meeting Abstract, Annual Meeting of the Society of Integrative and Comparative Biology.

van Linstow Otto. (1895). Untersuchungen an Nematoden. *Archiv Für Mikroskopische Anatomie*, *44*(1), 509–533. https://doi.org/10.1007/BF02934027

Van de Peer, Y., Ashman, T. -L., Soltis, P. S., & Soltis, D. E. (2021). Polyploidy: an evolutionary and ecological force in stressful times. *The Plant Cell*, *33*(1), 11–26. https://doi.org/10.1093/plcell/koaa015

Vandel (1928). La parthénogenèse géographique. Contribution à l'étude biologique et cytologique de la parthénogenèse naturelle. *Bulletin Biologique de La France et de La Belgique*, *62*, 164–281.

Vejdovsky, F. (1882). Über Drilophaga bucephalus, ein parasitisches Rädertier. *Sitzungsberichte Der Königlichen Böhmischen Gesellschaft Der Wissenschaften*, 390–397. https://www.zobodat.at/pdf/SB-Ges-Wiss-Prag_1882_0001-0492.pdf

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer International Publishing. https://doi.org/10.1007/978-3-319-24277-4

Wickham, H., & Bryan, J. (2023). *readxl: Read Excel Files* (R package version 1.4.3. https://CRAN.R-project.org/package=readxl). https://CRAN.R-project.org/package=readxl

Wickham, H., François, R., Henry, L., … Vaughan, D. (2023). *dplyr: A Grammar of Data Manipulation* (R package version 1.1.4). https://CRAN.R-project.org/package=dplyr

Wickham, H., Vaughan, D., & Girlich, M. (2024). *tidyr: Tidy Messy Data* (R package version 1.3.1). https://CRAN.R-project.org/package=tidyr

Downloaded from https://academic.oup.com/jeb/article/38/6/769/8119320 by NADEL user on 19 August 2025