Natural variation in *Caenorhabditis elegans* responses to the anthelmintic emodepside

Janneke Wit[a], Steffen R. Hahnel[a], Briana C. Rodriguez[a], and Erik. C. Andersen[a,‡]
[a]Molecular Biosciences, Northwestern University, Evanston, IL 60208

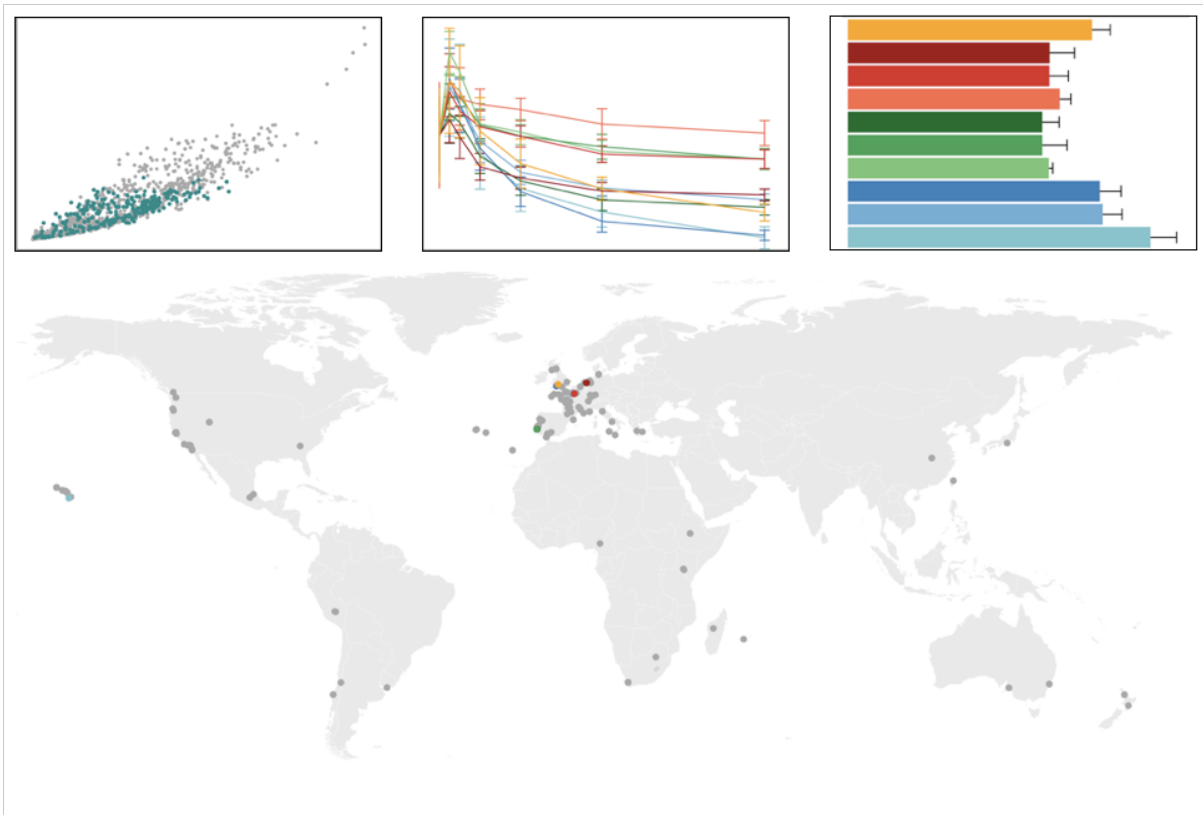
[‡]**Corresponding Author:**
Erik C. Andersen, Ph.D.
Department of Molecular Biosciences
Northwestern University
4619 Silverman Hall
2205 Tech Drive
Evanston, IL 60208
847-467-4382
erik.andersen@northwestern.edu

Janneke Wit: 0000-0002-3116-744X
Steffen R. Hahnel: 0000-0001-8848-0691
Briana C. Rodriguez: 0000-0002-5282-0815
Erik C. Andersen: 0000-0003-0229-9651

Journal: *IJP DDR*

Keywords: Emodepside, natural variation, *C. elegans*, anthelmintics, hormetic effect

Graphical abstract



Highlights (3-5, max 85 characters each):

- Emodepside responses vary across the *C. elegans* species.
- Wild strains of *C. elegans* model natural differences in parasite emodepside responses.
- Variation in the emodepside target *slo-1* and other loci correlate with resistance.
- Low doses of emodepside cause a hormetic effect on offspring production.

Abstract

Treatment of parasitic nematode infections depends primarily on the use of anthelmintics. However, this drug arsenal is limited, and resistance against most anthelmintics is widespread. Emodepside is a new anthelmintic drug effective against gastrointestinal and filarial nematodes. Nematodes that are resistant to other anthelmintic drug classes are susceptible to emodepside, indicating that the emodepside mode of action is distinct from previous anthelmintics. The laboratory-adapted *Caenorhabditis elegans* strain N2 is sensitive to emodepside, and genetic selection and *in vitro* experiments implicated *slo-1*, a BK potassium channel gene, in emodepside mode of action. In an effort to understand how natural populations will respond to emodepside, we measured brood sizes and developmental rates of wild *C. elegans* strains after exposure to the drug and found natural variation across the species. Some variation in emodepside responses can be explained by natural differences in *slo-1.* This result suggests that other genes in addition to *slo-1* underlie emodepside resistance in wild *C. elegans* strains. Additionally, all assayed strains have higher offspring production in low concentrations of emodepside (a hormetic effect), which could impact treatment strategies. We find that natural variation affects emodepside sensitivity, supporting the suitability of *C. elegans* as a model system to study emodepside responses across parasitic nematodes.

1. Introduction

Helminth infections are a major threat to animal and human health, and control measures depend heavily on a small arsenal of anthelmintic drugs. Resistance against most anthelmintic drug classes is widespread and documented for several species (McKellar and Jackson 2004; Kotze and Prichard 2016). New anthelmintics with a distinct mode of action can be used to treat populations resistant to multiple anthelmintics, but the introduction of new compounds is rare (Epe and Kaminsky 2013). One of the newest anthelmintics, the cyclooctadepsipeptide (COPD) emodepside, has been commercially available since 2007 (Epe and Kaminsky 2013). It is a synthetic derivative of a natural metabolite from the fungus *Mycelia sterilia* (Sasaki et al. 1992). As a broad spectrum anthelmintic, emodepside is efficacious against gastrointestinal nematodes and filarial nematodes (Achim Harder et al. 2003; Zahner et al. 2001) and is currently approved for treatment of helminth infections of cats and dogs in combination with praziquantel (Welz et al. 2011). No reports of field resistance have been reported since its introduction (Prichard 2017). Importantly, emodepside is effective against multi-drug resistant parasitic nematode strains, including ivermectin- and levamisole-resistant *Haemonchus contortus* (A. Harder et al. 2005; von Samson-Himmelstjerna et al. 2005).

Responses to emodepside have been studied in both parasitic nematodes and the free-living nematode *Caenorhabditis elegans*. Initial *in vitro* studies of the emodepside target using *Ascaris suum* suggested that the COPD PF1022A, the parent compound in emodepside synthesis (Jeschke et al. 2005), displaces GABAergic ligands from somatic muscle preparations (A. Harder et al. 2005). However, later work comparing the effect of GABA and emodepside on the rate of relaxation of contracted *A. suum* muscle showed that emodepside does not act directly on the GABAergic pathway (J. Willson et al. 2003; Willson J, Holden-Dye L, Harder A, Walker RJ 2001). Another promising lead was the identification of a putative target protein, HC110-R, from a *H. contortus* cDNA library (Saeger et al. 2001). Alignment revealed HC110-R had 48% identity and 76% similarity to the *C. elegans* latrophilin receptor LAT-1. Although predicted to be a heptahelical transmembrane protein, the exact function of HC110-R is unknown (Mühlfeld et al. 2009). Latrophilin is a G protein-coupled receptor in the secretin receptor family and a Ca^{2+}-independent receptor of alpha-latrotoxin (Welz et al. 2005). *C. elegans* larvae express *lat-1* in pharyngeal muscle, and adults express it in both pharyngeal and non-pharyngeal neurons (James Willson et al. 2004). In the laboratory strain N2, emodepside inhibits pharyngeal pumping, egg-laying, as well as locomotion (Bull et al. 2007). Putative null mutations in *lat-1* are less sensitive to emodepside-induced inhibition of pharyngeal pumping, but locomotor activity is inhibited (Guest et al. 2007; James Willson et al. 2004). This inhibition of locomotion suggests that emodepside affects pathways independent of *lat-1*.

A subsequent mutagenesis screen using *C. elegans* identified mutations in the Ca^{2+}-activated K^+ channel (BK-channel) gene *slo-1* in nine emodepside resistant mutants (Guest et al. 2007). These mutants were identified as highly resistant to inhibition of both pharyngeal pumping and locomotor activity by emodepside. Gain-of-function mutations in *slo-1*, like emodepside-treated nematodes, showed decreased locomotion and pharyngeal pumping (Davies et al. 2003) a putative *slo-1* null allele, *slo-1(js379)*, responded to emodepside treatment like mutants from the screen (Guest et al. 2007). Tissue-specific rescue experiments showed that emodepside inhibited locomotion by expression in both neurons and body wall muscle (Guest et al. 2007). However, feeding is inhibited through effects on pharyngeal-specific neurons alone and not through muscle. Emodepside has been shown to open SLO-1 channels expressed in *Xenopus laevis* oocytes (Kulke et al.

2014). Taken together, these results suggest that emodepside acts through a *slo-1* dependent pathway, and that the drug opens SLO-1 channels to inhibit locomotion and pharyngeal pumping.

The above studies on emodepside mode of action and resistance in *C. elegans* focused on the N2 laboratory strain and mutants in that genetic background. Although *C. elegans* is a great model organism for parasitic nematodes (Bürglin, Lobos, and Blaxter 1998; Dilks et al. 2020; Hahnel et al. 2018), studies that use only a single strain can be biased by rare variation or genetic modifiers specific to a single genetic background (Sterken et al. 2015). The observation that emodepside affects multiple nematode species suggests that its mode of action is conserved throughout the phylum. It is unlikely that one *C. elegans* strain represents all possible genes and variants that contribute to emodepside sensitivity. The use of multiple isolates in drug response studies increases the likelihood of elucidating mechanisms of resistance and drug mode of action shared by multiple strains and species (Hahnel et al. 2020; Wit, Dilks, and Andersen 2020). The natural variation across the *C. elegans* species is archived in the *C. elegans* Natural Diversity Resource (CeNDR) (Cook et al. 2017) and offers a powerful approach to look for genetic variation that underlies the different responses to emodepside, as has been done for other drugs (Evans and Andersen 2020; Brady et al. 2019; Hahnel et al. 2018; Zamanian et al. 2018; Zdraljevic et al. 2019, 2017).

Here, we measured emodepside responses in a set of *C. elegan*s wild strains to demonstrate that the effect of this anthelmintic on development and brood size depends on genetic background. Across a set of nine wild strains and the laboratory strain N2, we show that natural variation in *slo-1* is correlated with differences in response to emodepside, but additional variation impacts emodepside responses. This result illustrates the need for broader comparisons of anthelmintic resistance within a species, as variation in genes other than *slo-1* likely affects emodepside susceptibility. Additionally, it highlights the power of using *C. elegans* natural variation for studies of emodepside mode of action and resistance because this variation might recapitulate diversity present in parasite populations.

2. Materials and Methods

2.1 Strains

Animals were maintained at 20ºC on modified nematode growth medium (NGMA) containing 1% agar and 0.7% agarose seeded with the *E. coli* strain OP50 (Andersen et al. 2014). The laboratory strain N2 and a set of nine wild strains from the *C. elegans* Natural Diversity Resource (CeNDR) were used to study the response to multiple doses of emodepside and to determine the EC_{50}. Additionally, two *slo-1* putative loss-of-function mutant strains, BZ142 and NM1968, were obtained from the *Caenorhabditis* Genetics Center.

2.2 High-throughput fitness assays

The high-throughput fitness assays (HTAs) were performed using the COPAS BIOSORT (Union Biometrica, Holliston MA) as described previously (Zdraljevic et al. 2017; Hahnel et al. 2018). In summary, the strains were grown in uncrowded conditions to avoid the induction of dauer for four generations on NMGA plates at 20ºC prior to each assay. Gravid adults from the fifth generation were bleach-synchronized, and embryos were titered at one embryo per microliter of K medium (Boyd, Smith, and Freedman 2012) into 96-well microtiter plates and incubated overnight. Hatched L1 larvae were fed with 5 mg/mL HB101

145 lysate (Pennsylvania State University Shared Fermentation Facility, State College, PA
146 (García-González et al. 2017)) and cultured for 48 hours at 20ºC with constant shaking.
147 Three L4 larvae were then sorted into new microtiter plates containing modified K medium,
148 10 mg/mL HB101 lysate, 50 µM kanamycin, and either 1% DMSO or emodepside dissolved
149 in 1% DMSO.

150 After sorting, animals were cultured and allowed to reproduce for 96 hours at 20ºC
151 with constant shaking. For accurate nematode length measurements, the samples were
152 treated with sodium azide (50 mM in 1X M9) to straighten their bodies before analysis using
153 the COPAS BIOSORT. The COPAS BIOSORT is a large particle flow measurement device
154 (**Figure 1**), which measures time-of-flight (TOF), extinction (EXT), and fluorescence of
155 objects passing through the flow cell using laser beams. Animal length and optical density
156 measure nematode development because animals get longer as they progress through
157 development. If animals are negatively affected by emodepside, they are expected to be
158 smaller, less optically dense, and have smaller brood sizes. Animal optical density is
159 corrected for animal length (median.norm.EXT) for each object in each well. Object counts
160 are used to calculate brood size (norm.n), which is the number of objects passing the laser
161 corrected for the number of parent animals sorted into the well.

162 To determine concentrations to measure differences in emodepside responses
163 across the wild strains, a dose response assay was performed using three genetically
164 divergent *C. elegans* strains (N2, CB4856, and DL238) and four increasing concentrations of
165 emodepside (19.6, 39.1, 78.1, and 156.3 nM). A second dose response with 9.8, 19.6, 39.1,
166 78.1, 156.3, and 312.5 nM emodepside was performed using nine wild strains (JU751,
167 WN2001, NIC258, NIC265, NIC271, JU782, DL238, CB4932, and JU2586), two putative *slo-*
168 *1* null mutants (BZ142 and NM1968), and the N2 strain. These 12 strains were assayed in
169 six separate assays with four replicates in each assay. Raw phenotypic data were processed
170 for outliers and analyzed using the R package *easysorter* (Shimko and Andersen 2014) as
171 described previously (Hahnel et al. 2018). For each strain, all phenotypic values were
172 normalized by deducting the average trait value in control (DMSO) conditions.

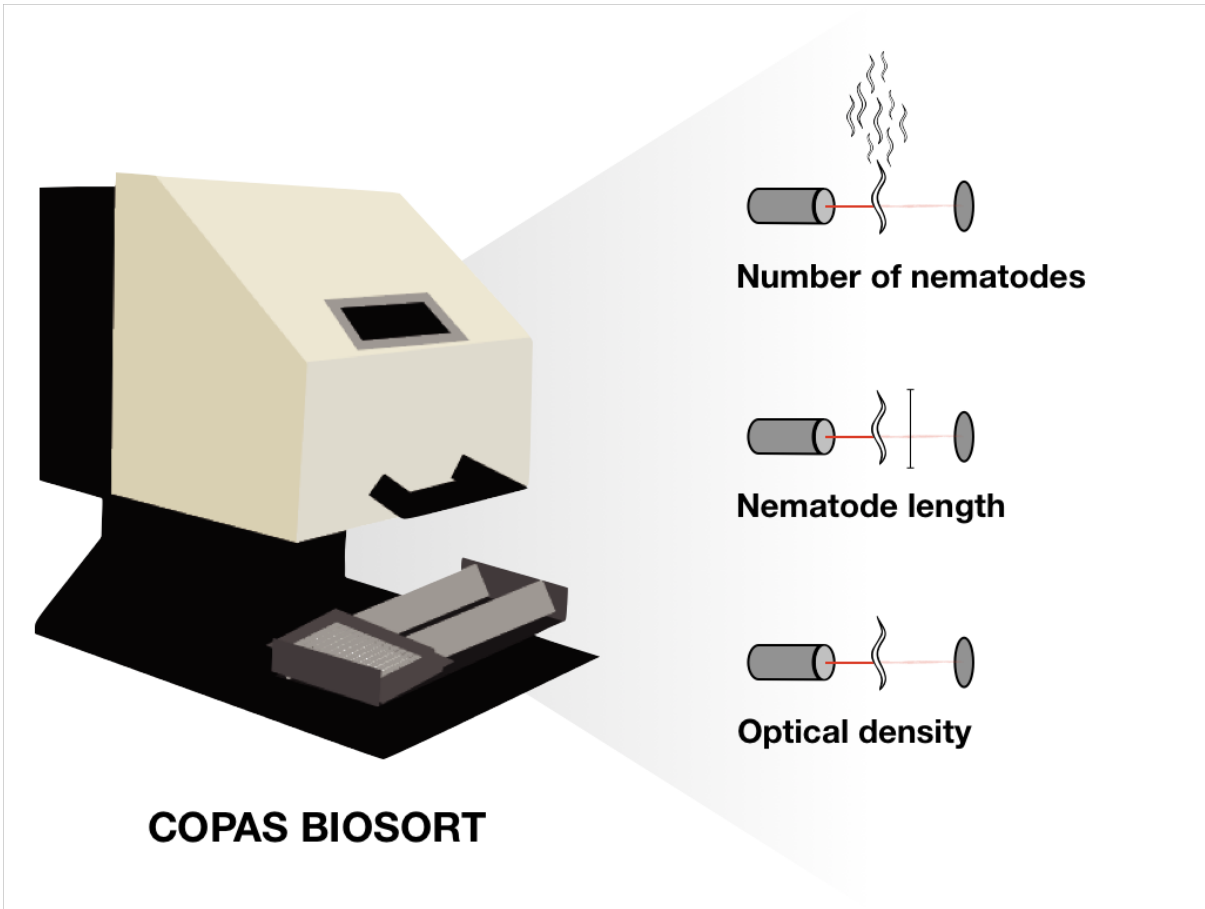


Figure 1
Using a COPAS BIOSORT, three independent traits were used to measure nematode responses to emodepside: brood size, nematode length (µm), and optical density.

2.3 Half maximal effective concentration (EC_{50}) calculations

To test if emodepside had an effect on any of the three traits across the range of concentrations, extreme outliers per dose were identified and removed if values were greater than or less than three times the interquartile range from the first or third quartile, respectively, using the identify_outliers from the R package *Rstatix* (Kassambara 2020). A Kruskal-Wallis test was performed for each strain (phenotype ~ dose) using the *rstatix* package (Kassambara 2020). For strains where emodepside had a phenotypic effect, the dose with the highest response was determined. To calculate the half maximal effective concentration (EC_{50}), the concentration at which 50% of the drug effect is reached, we could not simply fit from the control condition because of the hormetic effect (explained below). Instead, we fit a linear model (developmental trait or brood size ~ dose) to the data from the dose with the peak phenotypic value to the highest concentration (312.5 nM) assayed and calculated the concentration at the midpoint of the phenotypic effect. These EC_{50} values were calculated for each strain in each of the six assays and then the mean and standard deviation of each EC_{50} were calculated.

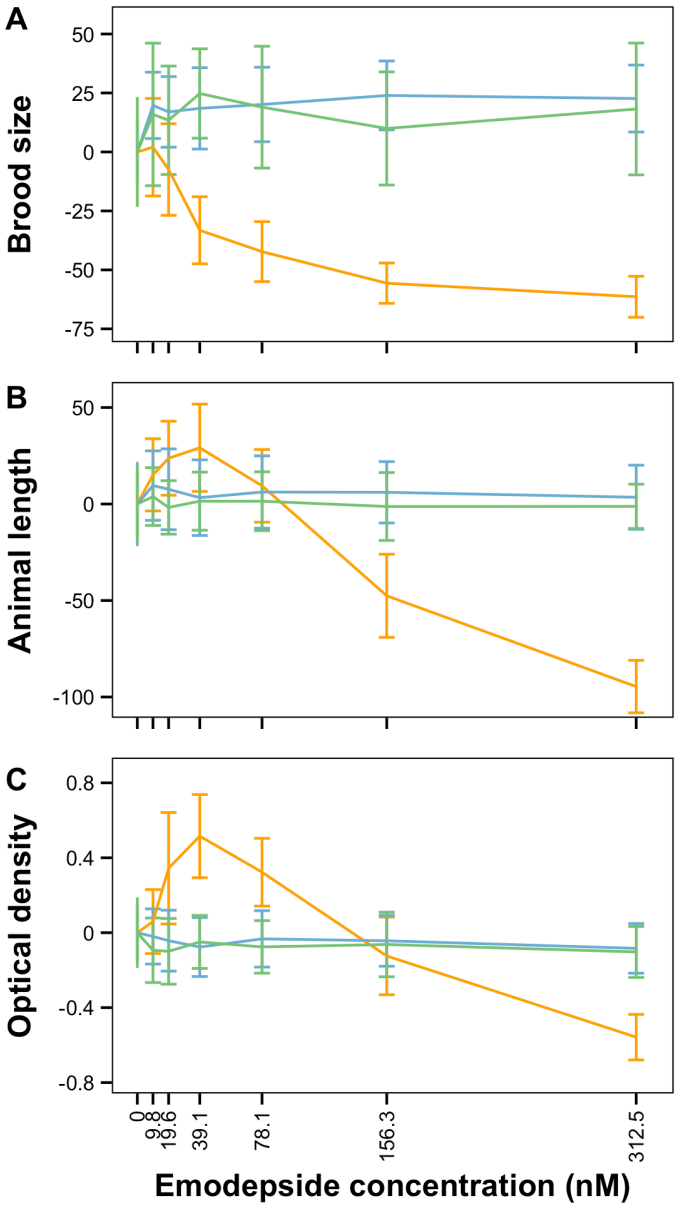
193 **2.4 Data availability**

194 **Supplementary File 1** contains the phenotypic values for N2 and two putative *slo-1*
195 null mutants in response to increasing concentrations of emodepside. **Supplementary File 2**
196 contains the phenotypic values for N2, CB4856, and DL238 in response to increasing
197 concentrations of emodepside. **Supplementary File 3** contains the raw extinction (EXT) and
198 time of flight (TOF) measurements for N2 and two wild *C. elegans* strains (CB4856 and
199 DL238) in control conditions and 78.1 nM emodepside. **Supplementary File 4** contains the
200 phenotypic values for the N2 strain and nine wild *C. elegans* strains in response to
201 increasing concentrations of emodepside. All data and scripts to generate figures can be
202 found at https://github.com/AndersenLab/emodepside_manuscript.
203
204 **3. Results**
205
206 **3.1 Putative *slo-1* null mutants are resistant to emodepside in the high-throughput**
207 **reproduction and development assays**

208 We assayed emodepside resistance as a function of nematode reproduction and
209 development. These traits were measured for thousands of animals using a previously
210 developed high-throughput assay (HTA) (see Methods, **Figure 1**) (Zdraljevic et al. 2019;
211 Brady et al. 2019; Evans et al. 2018; Zdraljevic et al. 2017; Andersen et al. 2015; Evans and
212 Andersen 2020; Hahnel et al. 2018; Zamanian et al. 2018; Evans et al. 2020). In this assay,
213 three L4 larvae were sorted into each well of a 96-well plate and allowed to grow and
214 reproduce for 96 hours in the presence of emodepside or DMSO. Each well contained these
215 three parents and hundreds of offspring. After 96 hours, animal length and optical density,
216 which are both proxies for nematode developmental stage (Andersen et al. 2015), were
217 measured for all progeny in the well. Animals grow longer and more dense over time, and
218 anthelmintics slow this development. Therefore, shorter and less optically dense animals
219 after 96 hours show that emodepside had a detrimental effect on development. In addition to
220 development, we also measured brood size as the average number of progeny produced
221 within the 96-hour window. Although ultimate brood size and demography of the population
222 influence statistical summaries of nematode development as measured by size (mean.TOF)
223 or optical density (median.norm.EXT), a smaller brood size shows emodepside sensitivity
224 (**Supplementary Figure 1**).

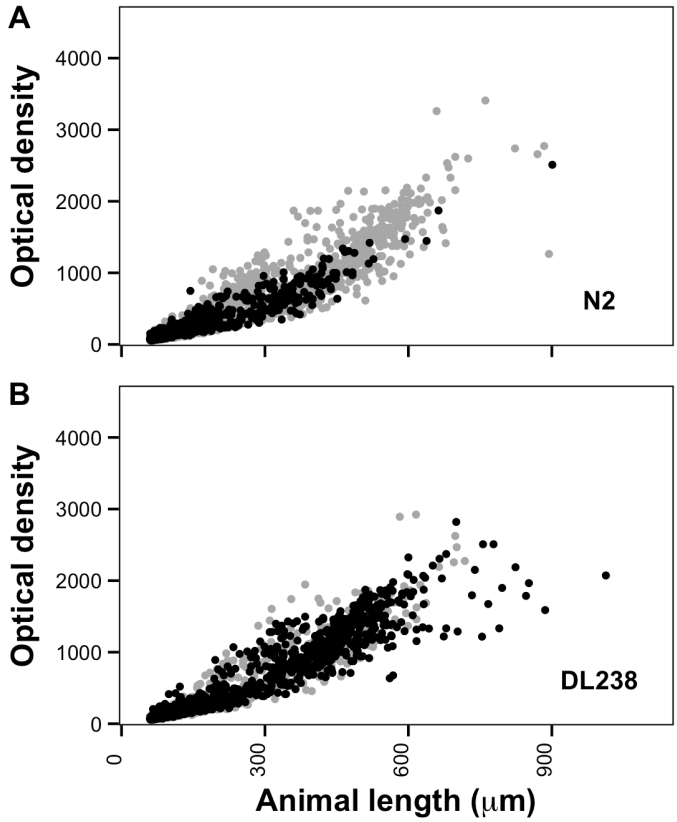
225 To confirm that our HTA could be used to quantitatively measure *C. elegans*
226 emodepside resistance, we measured animal development and brood size for two putative
227 *slo-1* null mutant strains, BZ142 and NM1968, and the laboratory strain, N2, across a range
228 of concentrations. The *slo-1* mutants were shown previously to be resistant to emodepside
229 based on locomotion and pharyngeal pumping assays (Guest et al. 2007), and the N2 strain
230 is known to be sensitive to emodepside. Brood size and development were both inhibited in
231 the N2 strain (Kruskal-Wallis, brood size: $p = 1.49 \times 10^{-42}$, animal length: $p = 4.32 \times 10^{-37}$,
232 optical density: $p = 1.25 \times 10^{-39}$), suggesting that the N2 strain is indeed sensitive to
233 emodepside in the HTA. Although development was not affected by emodepside for either
234 mutant strain (Kruskal-Wallis, BZ142 animal length: $p = 0.27$ and optical density: $p = 0.15$,
235 and NM1968 animal length: $p = 0.60$ and optical density: $p = 0.12$, **Figure 2,**
236 **Supplementary File 1**), the mutant strains both had higher brood sizes than the N2 strain in
237 emodepside (Kruskal-Wallis, BZ142: $p = 4.87 \times 10^{-11}$ and NM1968: $p = 6.97 \times 10^{-3}$). These
238 putative null mutant strains did not have significantly different brood sizes even at high
239 concentrations of emodepside (Kruskal-Wallis, brood size in increasing concentrations of
240 emodepside: BZ142, $p = 0.30$ and NM1968, $p = 0.26$). This result suggests that the mutant

241 strains are indeed resistant to emodepside. By contrast, both BZ142 and NM1968 had lower
242 brood sizes than the N2 strain in control conditions, showing that *slo-1* plays a role in
243 reproduction. Both deletion strains also had smaller average animal lengths and lower
244 optical densities than the N2 strain in control DMSO conditions (**Supplementary Figure 2,**
245 **Supplementary File 1**), which again demonstrates that the putative *slo-1* null mutants are
246 less fit than the N2 strain in control conditions. These results recapitulate previous studies
247 and illustrate the applicability of the HTA to study emodepside responses in *C. elegans*.



248 **Figure 2**
249 Dose response curves for (A) brood size, (B) animal length, and (C) optical density of the N2
250 strain (orange) and two *slo-1* putative null mutant strains (BZ142 = blue, NM1968 = green).
251
252 **3.2 Natural differences in emodepside response are heritable**
253 Previous studies of *C. elegans* resistance to emodepside have been conducted using
254 the N2 strain or mutant strains in the N2 genetic background. Assaying natural variation in *C.*
255 *elegans* was previously shown to be a powerful tool to identify genetic variation that
256 correlates with differences in benzimidazole responses (Hahnel et al. 2018). To test if the

257 response to emodepside varies by genetic background, we exposed a panel of three
258 genetically divergent *C. elegans* wild strains (N2, CB4856, and DL238) to increasing
259 concentrations of emodepside. At 78.1 nM emodepside, the phenotypic variation was
260 maximized among strains and minimized within replicates of the same strain as shown by
261 broad-sense heritabilities of 88% for brood size, 61% for animal length, and 60% for optical
262 density (**Supplementary Figure 3, Supplementary File 2**). At this concentration, N2
263 animals were both shorter and less optically dense in the presence of emodepside
264 compared to animals grown in control (DMSO) conditions, showing that development was
265 delayed (**Figure 3A, Supplementary File 3**). The CB4856 and DL238 strains were less
266 affected by this emodepside concentration (**Figure 3B and C, Supplementary File 3**).
267 These differential responses across the strains and the high heritabilities suggest that
268 genetic factors underlie natural variation in emodepside responses.



269 **Figure 3**
270 Plots of length and optical density values are shown for each nematode from (A) the
271 sensitive laboratory-adapted strain N2 strain and (B) a resistant wild strain from Hawaii
272 DL238. The gray points are nematodes grown in the presence of DMSO (control conditions),
273 and the black points are nematodes grown in the presence of DMSO and 78.1 nM
274 emodepside (emodepside conditions). *C. elegans* grows longer and more optically dense as
275 it ages, and anthelmintic effects can be measured as changes in the demography of animals
276 such that developmental delay is observed as smaller and less dense animals. The DL238
277 strain was resistant to emodepside because it grows equally well in both control and
278 emodepside conditions.

3.3 Emodepside affects brood size and development in a dose-dependent manner

To describe the effects of genetic background on development and brood size in response to emodepside in more detail, we selected ten genetically diverse wild strains for a second dose response assay to more highly replicate natural differences across the species. We detected significant variation in the dose-dependent responses to emodepside among strains (broad-sense heritabilities at 78.1 nM: 59.5% for brood size, 70.8% for animal length, and 70.8% for optical density). Interestingly, the sensitive laboratory strain N2 falls in the middle of this range, demonstrating that some wild strains are more susceptible to emodepside than the N2 strain and other strains are resistant (**Figure 4, Supplementary File 4**). At the highest concentration of 312.5 nM emodepside, development and brood size were inhibited for all strains. Although high concentrations of emodepside were shown to have detrimental effects on brood size and development, low concentrations of emodepside actually produced larger brood sizes compared to control conditions (**Figure 5, Supplementary Figure 4, Supplementary File 4**). This positive effect on fitness at low concentrations of the drug is called a hormetic effect (Bukowski and Lewis 2000)**.** For developmental traits the identification of a potential hormetic response is confounded by increased reproduction, because strains that develop further in low concentrations of emodepside start producing a second generation. This second generation increases the observed brood size, but also decreases the average length and optical density of the population because the next generation of early larval stage animals are short and not optically dense (**Supplementary Figure 1**). Regardless of the presence of a hormetic effect for development, the increased brood size at low doses of emodepside suggests emodepside causes a hormetic effect.

We next calculated the concentration with half of the maximal drug effect (EC_{50}) for each of the strains and all three traits (**Figure 4 D-F, Supplementary File 4**). For all traits, the EC_{50} was significantly affected by genetic diversity across the strains that were assayed (Kruskal-Wallis, brood size: $p = 0.0439$, animal length: $p = 8.01 \times 10^{-8}$, optical density: $p = 2.14 \times 10^{-7}$). Overall, these results demonstrate that natural variation in *C. elegans* affects the emodepside response, and that this model provides an excellent system to study the genetics of emodepside mode of action and resistance.

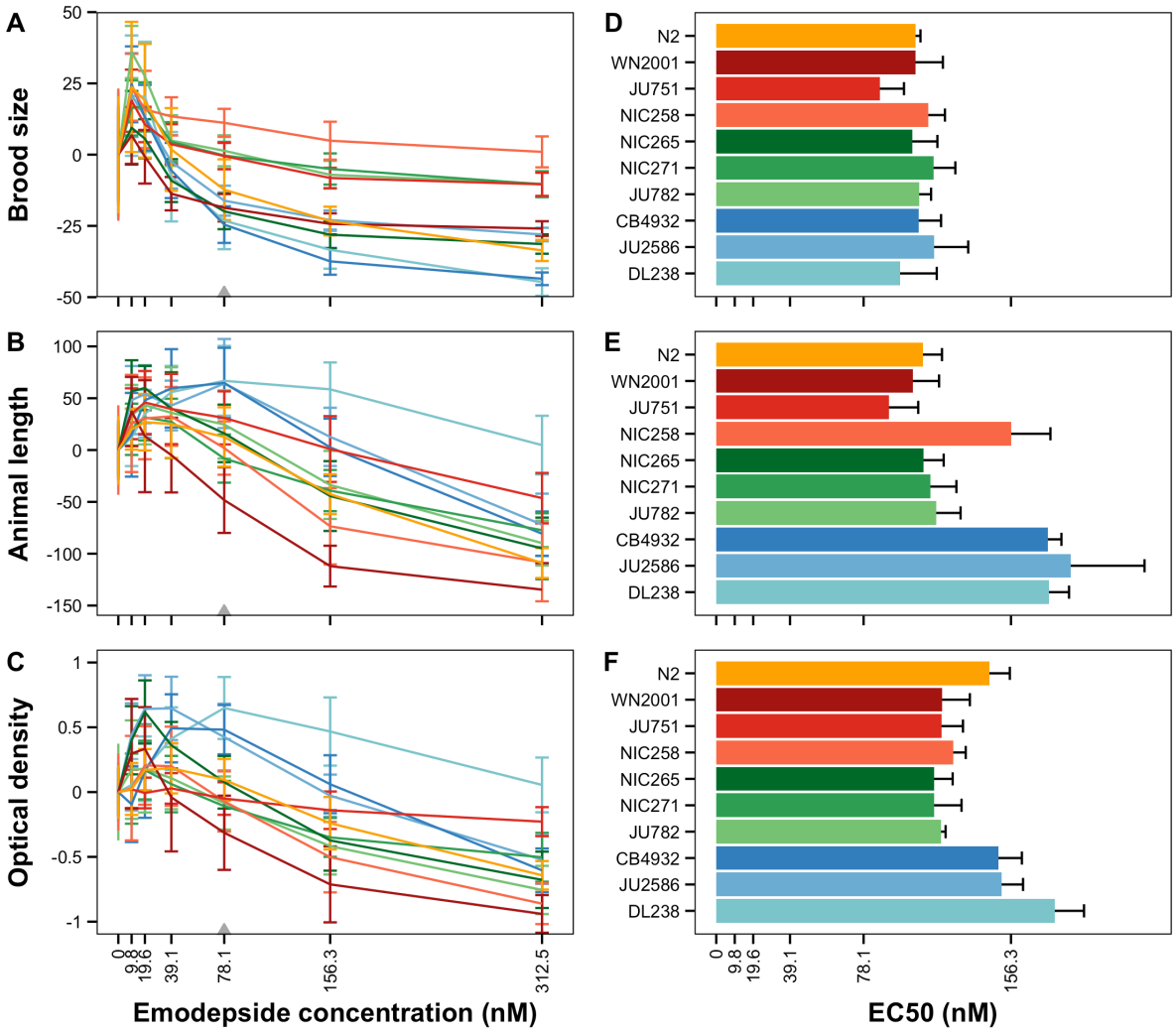


Figure 4

Dose response curves of nine wild *C. elegans* strains and N2 of (A) brood size, (B) animal length, and (C) optical density are shown on the left. Average EC_{50} values per strain for (D) brood size, (E) animal length, and (F) optical density are shown on the right.

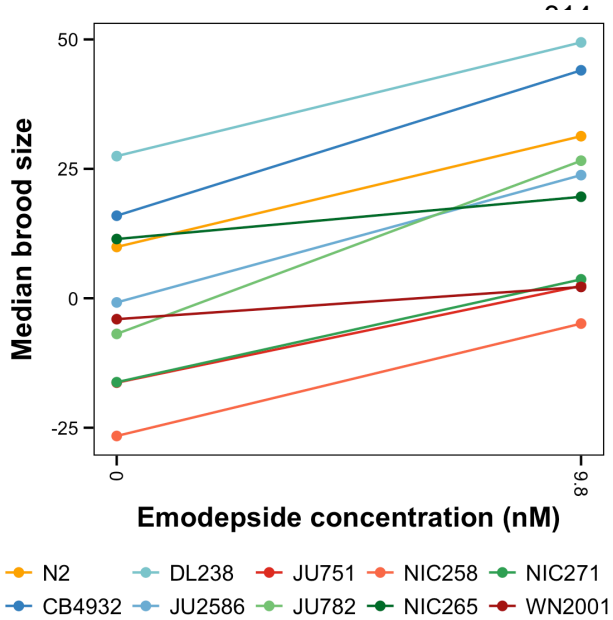


Figure 5

Plot of median brood sizes at the control condition (DMSO) and at 9.8 nM emodepside for ten *C. elegans* strains. Statistical significance of the phenotypic response in DMSO compared to 9.8 nM emodepside was calculated using a pairwise Wilcoxon test. All strains, except NIC265, showed a significant ($p < 0.05$) hormetic effect.

325 **3.4 Natural variation in candidate gene *slo-1* correlates with resistance in**
326 **reproduction but not development in wild C. elegans strains**
327 Emodepside has been shown to directly interact with and open the *C. elegans* SLO-1
328 channel (Kulke et al. 2014), and putative *slo-1* null mutants are resistant to emodepside
329 treatment (**Figure 2**, **Supplementary File 1** (Guest et al. 2007)). Because of these results,
330 we expected that deleterious variation in *slo-1* in the wild strains would correlate with
331 emodepside resistance. Of nine wild strains assayed here, four (NIC258, NIC265, NIC271,
332 and JU782) have the same variation in *slo-1* (Arg134Trp, Leu327Phe, Cys328Leu, and
333 Arg678Leu) that causes deleterious amino acid substitutions with a summed BLOSUM score
334 of -6 (Henikoff and Henikoff 1992). This variation correlated with higher EC_{50} values for
335 brood size but lower EC_{50} values for development (Kruskal-Wallis, brood size: $p = 0.0221$,
336 animal length: $p = 0.263$, optical density: $p = 3.35x10^{-5}$), which indicates that these strains
337 are resistant to emodepside. This opposing trend between brood size and development is
338 the result of an increase in reproduction as described above.
339 We also investigated natural variation in another candidate gene for emodepside
340 resistance, *lat-1* (James Willson et al. 2004; Saeger et al. 2001). All nine wild strains in the
341 dose response assay harbor natural variants in *lat-1*. To investigate if that variation is
342 predicted to be deleterious to *lat-1* function, we summed BLOSUM scores for each of the
343 wild strains. Only the DL238 strain had a negative BLOSUM score (-1), and this score was
344 not correlated with resistance across all strains (Kruskal-Wallis, brood size: $p = 0.223$,
345 animal length: $p = 0.223$, and optical density: $p = 0.117$). In this set of ten wild strains,
346 variation in *lat-1* does not underlie differences in emodepside responses. Because strains
347 vary in emodepside responses outside the range of these strains, our results show that
348 variation in *slo-1* or *lat-1* does not explain all differences in emodepside responses,
349 suggesting that additional genes affect the response to emodepside.
350
351 **4. Discussion**
352 Emodepside is a broad range anthelmintic with a distinct mode of action compared to
353 other anthelmintics (Epe and Kaminsky 2013). Previous studies of emodepside sensitivity
354 and phenotypic effects in *C. elegans* have focussed on the laboratory strain N2 (Bull et al.
355 2007; James Willson et al. 2004; Guest et al. 2007). In this strain, emodepside inhibits egg-
356 laying, pharyngeal pumping, development, and locomotion. In the present study, sensitivity
357 to emodepside was measured across wild *C. elegans* strains, the N2 strain, and two putative
358 *slo-1* null mutants (BZ142 and NM1968) using a large-particle flow cytometer high-
359 throughput assay (HTA) (**Figure 1**). Resistance to emodepside caused by the putative *slo-1*
360 mutants was confirmed using the HTA. Additionally, the effects of emodepside on brood size
361 and development varied across the wild strains (**Figures 3 and 4, Supplementary Files 3**
362 **and 4**) and was correlated with protein-coding variation in the resistance candidate gene *slo-*
363 *1*. Importantly, we found that low doses of emodepside have a hormetic effect on brood size.
364 Hormesis was observed in all nine wild strains and the N2 laboratory strain, regardless of
365 their susceptibility to emodepside (**Figure 4, Supplementary File 4**). This consistent
366 hormetic effect suggests that emodepside might also cause a hormetic effect in parasitic
367 nematodes. This study shows the power of using natural variation in *C. elegans* to study
368 emodepside responses.

369 **4.1 High-throughput assays across wild strains show the effects of emodepside on**
370 **development and reproduction**
371 In the present study, development and reproductive success in the presence of
372 emodepside was measured for the N2 strain and a set of wild *C. elegans* strains using a
373 HTA. Previously, reproduction was measured on agar plates (Bull et al. 2007), where both
374 the timing and the quantity of egg laying was measured. Using the HTA, brood size is
375 assayed as the overall reproductive success of three L4 larvae over a 96-hour period.
376 Results from agar-based and the HTA are highly correlated, and HTA intra- and inter-assay
377 correlations are substantially greater compared to agar plate-based methods (Andersen et
378 al. 2015). On agar plates, emodepside prevents egg laying, and the animals are bloated with
379 embryos at higher drug concentrations (20 nM - 500 nM) (Bull et al. 2007). In the HTA,
380 reproduction was inhibited for the N2 strain as well as the wild strains at concentrations
381 similar to previous studies (19.6 μM to 312.5 nM), confirming that brood size in response to
382 emodepside can be measured reproducibly with both assays. Agar-based developmental
383 rate, based on the percentage of eggs that hatch and reach different larval stages in
384 increasing concentrations of emodepside, is delayed (Bull et al. 2007). The HTA measures
385 animal length and optical density of a population established by three L4 larvae over a 96-
386 hour period. The two lowest concentrations of emodepside, 9.8 nM and 19.8 nM, have either
387 no effect on development or a hormetic effect. Higher concentrations (39.1 μM to 312.5 nM),
388 which overlap with the effective concentrations from the agar-based development
389 phenotypes, negatively affect animal length and optical density (**Figure 4**). The results from
390 both the agar-based and HTA methods indicate that emodepside inhibits reproduction at
391 lower concentrations than development. Emodepside inhibited reproduction from
392 approximately 20 nM and up, compared to approximately 40 nM and up for development.
393 The agar-based study did not find a hormetic effect, but our results suggest that such an
394 effect is likely to be present at concentrations below the range tested on agar plates. Our
395 results show that the HTA provides a platform to screen hundreds of strains efficiently and
396 that the different measures of reproduction and development are similarly affected across
397 assay platforms.
398
399 **4.2 Natural variation affects development and brood size in the presence of**
400 **emodepside**
401 The response of *C. elegans* to emodepside is affected by natural genetic variation
402 (**Figure 4, Supplementary File 4**). Our results showed that all strains are affected by
403 emodepside, and that higher doses inhibit development, as measured by animal length and
404 optical density, and reproduction, as measured by brood size (**Figure 4, Supplementary**
405 **File 4**). For brood size, strain-specific differences were correlated with variation in *slo-1*
406 where strains with predicted deleterious variation were more resistant to emodepside
407 treatments. However, strains with higher brood sizes at lower concentrations do not have
408 variation in *slo-1*, suggesting that the hormetic effect is not mediated by *slo-1*. It will be
409 informative to introduce *slo-1* variation in the wild strains with higher fitness at lower
410 concentrations using CRISPR-Cas9 genome editing to test if *slo-1* variation reduces brood
411 size in a more resistant background. Our results show that reproduction and development
412 are inhibited by higher concentrations of emodepside, and that natural variation affects the
413 extent of this response. Future measurements of emodepside responses across additional
414 wild isolates will improve the power to detect candidate resistance genes across the species
415 using genome-wide association studies.

416 **4.3 Hormetic effects of emodepside suggest a potential risk of treatment failure at**
417 **suboptimal doses**
418 All ten strains showed a hormetic response in reproduction (**Figure 4 A-C, Figure 5,**
419 **Supplementary File 4**). The presence of hormetic responses across strains illustrates that
420 hormesis is a common response to low concentrations of emodepside across *C. elegans*
421 strains. If this response is shared with parasitic nematodes, then experimental designs to
422 calculate EC_{50} values in parasites need to account for this effect. An EC_{50} with a lower value
423 caused by a hormetic effect can lead to recommended treatment doses that will be
424 insufficient to treat parasitic nematode infections. Underdosing is a known risk factor for the
425 selection of resistance against all anthelmintics (Sangster, Cowling, and Woodgate 2018;
426 Smith et al. 1999; Silvestre, Cabaret, and Humbert 2001). If hormetic doses are
427 administered, either because of erroneous EC_{50} calculations or as a result of other treatment
428 factors like ineffective drug delivery (Sangster, Cowling, and Woodgate 2018), the infection
429 might be intensified rather than treated. Our results also imply that low doses of emodepside
430 are beneficial rather than detrimental for nematode growth. To prevent hormesis from
431 rendering emodepside treatment ineffective, it is essential to investigate hormetic effects and
432 adjust treatment recommendations accordingly.
433
434 **4.4 Natural variation in *C. elegans* can facilitate the study of anthelmintic resistance**
435 The free-living nematode *C. elegans* is a long standing model to study anthelmintic
436 mode of action and resistance of parasitic nematodes (Wit, Dilks, and Andersen 2020;
437 Holden-Dye et al. 2014; Geary and Thompson 2001). The suitability of *C. elegans* as a
438 model is the result of a range of attributes, including the phylogenetic relationship of *C.*
439 *elegans* with many parasitic nematodes of human and veterinary importance, its short and
440 direct life cycle, a wide range of genome-editing tools, and its high-quality reference genome
441 and gene models. Additionally, larval stages of many parasitic nematodes occupy the same
442 niches as *C. elegans* (Frézal and Félix 2015; Crombie et al. 2019). Similar environmental
443 stressors, including naturally occuring precursors of anthelmintics (Campbell 2012, 2005;
444 Alivisatos, Lamantia, and Matijevitch 1962), can cause similar selective pressures for both
445 species to evolve resistance.
446 Previous studies on the emodepside resistance candidate gene *lat-1*, showed that
447 putative *lat-1* null mutants were resistant in reproduction and pharyngeal pumping assays,
448 but sensitive in locomotion assays (Guest et al. 2007). Here, we show that although putative
449 *slo-1* null mutants are resistant to anthelmintic treatment, *slo-1* variation is not the only
450 determinant of resistance across wild strains. These results imply that multiple genes likely
451 affect the response to emodepside. To identify these genes, genetic variation across wild
452 strains can be correlated with phenotypic responses to emodepside. Genes identified based
453 on population-wide variation are more likely to translate to other species than genes
454 identified based on one genetic background. After identification of candidate genes, genetic
455 variation in these genes should be tested in a controlled genetic background by introducing
456 specific mutations using CRISPR-Cas9 genome editing (Dilks et al. 2020).
457
458 **Declaration of competing interests**
459 The authors have no competing financial or personal interests that impacted the work
460 presented in this manuscript.

Acknowledgements

We would also like to thank members of the Andersen laboratory for their technical assistance and helpful comments on the manuscript. S.R.H. was funded by a DFG fellowship (HA 8449/1-1) from the Deutsche Forschungsgemeinschaft. This work was supported by R01 AI153088 to E.C.A. We would also like to thank Wormbase and the *C. elegans* Natural Diversity Resource (CeNDR) for data and tools critical for our analyses of natural variation (NSF CSBR 1930382). The *slo-1* mutant strains (BZ142 and NM1968) were provided by the *Caenorhabditis* Genetics Center, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440).

470 **References**

471 Alivisatos, S. G., L. Lamantia, and B. L. Matijevitch. 1962. “Imidazolytic Processes. VI.
472 Enzymic Formation of Benzimidazole and 5,6-Dimethylbenzimidazole Containing
473 Dinucleotides.” *Biochimica et Biophysica Acta* 58 (April): 209–17.
474 Andersen, Erik C., Joshua S. Bloom, Justin P. Gerke, and Leonid Kruglyak. 2014. “A Variant
475 in the Neuropeptide Receptor Npr-1 Is a Major Determinant of Caenorhabditis Elegans
476 Growth and Physiology.” *PLoS Genetics* 10 (2): e1004156.
477 Andersen, Erik C., Tyler C. Shimko, Jonathan R. Crissman, Rajarshi Ghosh, Joshua S.
478 Bloom, Hannah S. Seidel, Justin P. Gerke, and Leonid Kruglyak. 2015. “A Powerful New
479 Quantitative Genetics Platform, Combining Caenorhabditis Elegans High-Throughput
480 Fitness Assays with a Large Collection of Recombinant Strains.” *G3* 5 (5): 911–20.
481 Boyd, Windy A., Marjolein V. Smith, and Jonathan H. Freedman. 2012. “Caenorhabditis
482 Elegans as a Model in Developmental Toxicology.” *Methods in Molecular Biology*.
483 https://doi.org/10.1007/978-1-61779-867-2_3.
484 Brady, Shannon C., Stefan Zdraljevic, Karol W. Bisaga, Robyn E. Tanny, Daniel E. Cook,
485 Daehan Lee, Ye Wang, and Erik C. Andersen. 2019. “A Novel Gene Underlies
486 Bleomycin-Response Variation in Caenorhabditis Elegans.” *Genetics* 212 (4): 1453–68.
487 Bukowski, J. A., and R. J. Lewis. 2000. “Hormesis and Health: A Little of What You Fancy
488 May Be Good for You.” *Southern Medical Journal* 93 (4): 371–74.
489 Bull, Kathryn, Alan Cook, Neil A. Hopper, Achim Harder, Lindy Holden-Dye, and Robert J.
490 Walker. 2007. “Effects of the Novel Anthelmintic Emodepside on the Locomotion, Egg-
491 Laying Behaviour and Development of Caenorhabditis Elegans.” *International Journal*
492 *for Parasitology* 37 (6): 627–36.
493 Bürglin, Thomas R., Edgar Lobos, and Mark L. Blaxter. 1998. “Caenorhabditis Elegans as a
494 Model for Parasitic Nematodes.” *International Journal for Parasitology*.
495 https://doi.org/10.1016/s0020-7519(97)00208-7.
496 Campbell, William C. 2005. “Serendipity and New Drugs for Infectious Disease.” *ILAR*
497 *Journal / National Research Council, Institute of Laboratory Animal Resources* 46 (4):
498 352–56.
499 ———. 2012. “History of Avermectin and Ivermectin, with Notes on the History of Other
500 Macrocyclic Lactone Antiparasitic Agents.” *Current Pharmaceutical Biotechnology* 13
501 (6): 853–65.
502 Cook, Daniel E., Stefan Zdraljevic, Joshua P. Roberts, and Erik C. Andersen. 2017.
503 “CeNDR, the Caenorhabditis Elegans Natural Diversity Resource.” *Nucleic Acids*
504 *Research* 45 (D1): D650–57.
505 Crombie, Tim A., Stefan Zdraljevic, Daniel E. Cook, Robyn E. Tanny, Shannon C. Brady, Ye
506 Wang, Kathryn S. Evans, et al. 2019. “Deep Sampling of Hawaiian Caenorhabditis
507 Elegans Reveals High Genetic Diversity and Admixture with Global Populations.” *eLife* 8
508 (December): e50465.
509 Davies, Andrew G., Jonathan T. Pierce-Shimomura, Hongkyun Kim, Miri K. VanHoven, Tod
510 R. Thiele, Antonello Bonci, Cornelia I. Bargmann, and Steven L. McIntire. 2003. “A
511 Central Role of the BK Potassium Channel in Behavioral Responses to Ethanol in C.
512 Elegans.” *Cell*. https://doi.org/10.1016/s0092-8674(03)00979-6.
513 Dilks, C. M., S. Hahnel, Q. Sheng, L. Long, and P. T. McGrath. 2020. “Quantitative
514 Benzimidazole Resistance and Fitness Effects of Parasitic Nematode Beta-Tubulin
515 Alleles.” *BioRxiv*.
516 https://www.biorxiv.org/content/10.1101/2020.07.07.191866v1.abstract.

517 Driscoll, M., E. Dean, E. Reilly, E. Bergholz, and M. Chalfie. 1989. “Genetic and Molecular
518 Analysis of a Caenorhabditis Elegans Beta-Tubulin That Conveys Benzimidazole
519 Sensitivity.” *The Journal of Cell Biology* 109 (6 Pt 1): 2993–3003.
520 Epe, Christian, and Ronald Kaminsky. 2013. “New Advancement in Anthelmintic Drugs in
521 Veterinary Medicine.” *Trends in Parasitology* 29 (3): 129–34.
522 Evans, Kathryn S., and Erik C. Andersen. 2020. “The Gene Scb-1 Underlies Variation in
523 Caenorhabditis Elegans Chemotherapeutic Responses.” *G3* , May.
524 https://doi.org/10.1534/g3.120.401310.
525 Evans, Kathryn S., Shannon C. Brady, Joshua S. Bloom, Robyn E. Tanny, Daniel E. Cook,
526 Sarah E. Giuliani, Stephen W. Hippleheuser, Mostafa Zamanian, and Erik C. Andersen.
527 2018. “Shared Genomic Regions Underlie Natural Variation in Diverse Toxin
528 Responses.” *Genetics*, October. https://doi.org/10.1534/genetics.118.301311.
529 Evans, Kathryn S., Stefan Zdraljevic, Lewis Stevens, Kimberly Collins, Robyn E. Tanny, and
530 Erik C. Andersen. 2020. “Natural Variation in the Sequestosome-Related Gene, Sqst-5,
531 Underlies Zinc Homeostasis in Caenorhabditis Elegans.” *PLoS Genetics* 16 (11):
532 e1008986.
533 Fleming, J. T., M. D. Squire, T. M. Barnes, C. Tornoe, K. Matsuda, J. Ahnn, A. Fire, et al.
534 1997. “Caenorhabditis Elegans Levamisole Resistance Genes Lev-1, Unc-29, and Unc-
535 38 Encode Functional Nicotinic Acetylcholine Receptor Subunits.” *The Journal of*
536 *Neuroscience: The Official Journal of the Society for Neuroscience* 17 (15): 5843–57.
537 Frézal, Lise, and Marie-Anne Félix. 2015. “C. Elegans Outside the Petri Dish.” *eLife* 4
538 (March). https://doi.org/10.7554/eLife.05849.
539 García-González, Aurian P., Ashlyn D. Ritter, Shaleen Shrestha, Erik C. Andersen, L. Safak
540 Yilmaz, and Albertha J. M. Walhout. 2017. “Bacterial Metabolism Affects the C. Elegans
541 Response to Cancer Chemotherapeutics.” *Cell*.
542 https://doi.org/10.1016/j.cell.2017.03.046.
543 Geary, Timothy G., and David P. Thompson. 2001. “Caenorhabditis Elegans: How Good a
544 Model for Veterinary Parasites?” *Veterinary Parasitology*. https://doi.org/10.1016/s0304-
545 4017(01)00562-3.
546 Guest, Marcus, Kathryn Bull, Robert J. Walker, Kiran Amliwala, Vincent O’Connor, Achim
547 Harder, Lindy Holden-Dye, and Neil A. Hopper. 2007. “The Calcium-Activated
548 Potassium Channel, SLO-1, Is Required for the Action of the Novel Cyclo-
549 Octadepsipeptide Anthelmintic, Emodepside, in Caenorhabditis Elegans.” *International*
550 *Journal for Parasitology* 37 (14): 1577–88.
551 Hahnel, Steffen R., Clayton M. Dilks, Iring Heisler, Erik C. Andersen, and Daniel Kulke.
552 2020. “Caenorhabditis Elegans in Anthelmintic Research – Old Model, New
553 Perspectives.” *International Journal for Parasitology: Drugs and Drug Resistance*.
554 https://doi.org/10.1016/j.ijpddr.2020.09.005.
555 Hahnel, Steffen R., Stefan Zdraljevic, Briana C. Rodriguez, Yuehui Zhao, Patrick T.
556 McGrath, and Erik C. Andersen. 2018. “Extreme Allelic Heterogeneity at a
557 Caenorhabditis Elegans Beta-Tubulin Locus Explains Natural Resistance to
558 Benzimidazoles.” *PLoS Pathogens* 14 (10): e1007226.
559 Harder, Achim, Hans-Peter Schmitt-Wrede, Jürgen Krücken, Predrag Marinovski, Frank
560 Wunderlich, James Willson, Kiran Amliwala, Lindy Holden-Dye, and Robert Walker.
561 2003. “Cyclooctadepsipeptides—an Anthelmintically Active Class of Compounds
562 Exhibiting a Novel Mode of Action.” *International Journal of Antimicrobial Agents* 22 (3):
563 318–31.
564 Harder, A., L. Holden-Dye, R. Walker, and F. Wunderlich. 2005. “Mechanisms of Action of

Emodepside." *Parasitology Research* 97 Suppl 1 (October): S1–10.
Henikoff, S., and J. G. Henikoff. 1992. "Amino Acid Substitution Matrices from Protein Blocks." *Proceedings of the National Academy of Sciences of the United States of America* 89 (22): 10915–19.
Holden-Dye, Lindy, Southampton Neuroscience Group (SoNG), Centre for Biological Sciences, University of Southampton, Southampton So17 1bj, UK., and Robert J. Walker. 2014. "Anthelmintic Drugs and Nematicides: Studies in Caenorhabditis Elegans." *WormBook*. https://doi.org/10.1895/wormbook.1.143.2.
Jeschke, R., K. Iinuma, A. Harder, M. Schindler, and T. Murakami. 2005. "Influence of the Cyclooctadepsipeptides PF1022A and PF1022E as Natural Products on the Design of Semi-Synthetic Anthelmintics such as Emodepside." *Parasitology Research* 97 Suppl 1 (October): S11–16.
Kaminsky, Ronald, Pierre Ducray, Martin Jung, Ralph Clover, Lucien Rufener, Jacques Bouvier, Sandra Schorderet Weber, et al. 2008. "A New Class of Anthelmintics Effective against Drug-Resistant Nematodes." *Nature* 452 (7184): 176–80.
Kassambara, A. 2020. "Rstatix: Pipe-Friendly Framework for Basic Statistical Tests. R Package Version 0.4. 0." Avaliable online at: https://cran. r-project. org/web/packages/rstatix/index ….
Kotze, A. C., and R. K. Prichard. 2016. "Anthelmintic Resistance in Haemonchus Contortus: History, Mechanisms and Diagnosis." *Advances in Parasitology* 93 (March): 397–428.
Kulke, Daniel, Georg von Samson-Himmelstjerna, Sandra M. Miltsch, Adrian J. Wolstenholme, Aaron R. Jex, Robin B. Gasser, Cristina Ballesteros, et al. 2014. "Characterization of the Ca2+-Gated and Voltage-Dependent K+-Channel Slo-1 of Nematodes and Its Interaction with Emodepside." *PLoS Neglected Tropical Diseases* 8 (12): e3401.
McKellar, Quintin A., and Frank Jackson. 2004. "Veterinary Anthelmintics: Old and New." *Trends in Parasitology* 20 (10): 456–61.
Mühlfeld, Stefanie, Hans-Peter Schmitt-Wrede, Achim Harder, and Frank Wunderlich. 2009. "FMRFamide-like Neuropeptides as Putative Ligands of the Latrophilin-like HC110-R from Haemonchus Contortus." *Molecular and Biochemical Parasitology* 164 (2): 162–64.
Prichard, Roger K. 2017. "Drug Resistance in Nematodes." In *Antimicrobial Drug Resistance: Mechanisms of Drug Resistance, Volume 1*, edited by Douglas L. Mayers, Jack D. Sobel, Marc Ouellette, Keith S. Kaye, and Dror Marchaim, 689–704. Cham: Springer International Publishing.
Saeger, B., H. P. Schmitt-Wrede, M. Dehnhardt, W. P. Benten, J. Krücken, A. Harder, G. Von Samson-Himmelstjerna, H. Wiegand, and F. Wunderlich. 2001. "Latrophilin-like Receptor from the Parasitic Nematode Haemonchus Contortus as Target for the Anthelmintic Depsipeptide PF1022A." *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology* 15 (7): 1332–34.
Samson-Himmelstjerna, G. von, A. Harder, N. C. Sangster, and G. C. Coles. 2005. "Efficacy of Two Cyclooctadepsipeptides, PF1022A and Emodepside, against Anthelmintic-Resistant Nematodes in Sheep and Cattle." *Parasitology* 130 (Pt 3): 343–47.
Sangster, Nicholas C., Ann Cowling, and Robert G. Woodgate. 2018. "Ten Events That Defined Anthelmintic Resistance Research." *Trends in Parasitology* 34 (7): 553–63.
Sasaki, T., M. Takagi, T. Yaguchi, S. Miyadoh, T. Okada, and M. Koyama. 1992. "A New Anthelmintic Cyclodepsipeptide, PF1022A." *The Journal of Antibiotics* 45 (5): 692–97.
Shimko, Tyler C., and Erik C. Andersen. 2014. "COPASutils: An R Package for Reading, Processing, and Visualizing Data from COPAS Large-Particle Flow Cytometers." *PloS*

613 *One* 9 (10): e111090.
614 Silvestre, A., J. Cabaret, and J. F. Humbert. 2001. “Effect of Benzimidazole under-Dosing on
615 the Resistant Allele Frequency in Teladorsagia Circumcincta (Nematoda).” *Parasitology*
616 123 (Pt 1): 103–11.
617 Smith, G., B. T. Grenfell, V. Isham, and S. Cornell. 1999. “Anthelmintic Resistance Revisited:
618 Under-Dosing, Chemoprophylactic Strategies, and Mating Probabilities.” *International*
619 *Journal for Parasitology* 29 (1): 77–91; discussion 93–94.
620 Sterken, Mark G., L. Basten Snoek, Jan E. Kammenga, and Erik C. Andersen. 2015. “The
621 Laboratory Domestication of Caenorhabditis Elegans.” *Trends in Genetics: TIG* 31 (5):
622 224–31.
623 Welz, C., A. Harder, T. Schnieder, J. Hoglund, and G. von Samson-Himmelstjerna. 2005.
624 “Putative G Protein-Coupled Receptors in Parasitic Nematodes--Potential Targets for
625 the New Anthelmintic Class Cyclooctadepsipeptides?” *Parasitology Research* 97 Suppl
626 1 (October): S22–32.
627 Welz, Claudia, Nina Krüger, Monika Schniederjans, Sandra M. Miltsch, Jürgen Krücken,
628 Marcus Guest, Lindy Holden-Dye, Achim Harder, and Georg von Samson-
629 Himmelstjerna. 2011. “SLO-1-Channels of Parasitic Nematodes Reconstitute Locomotor
630 Behaviour and Emodepside Sensitivity in Caenorhabditis Elegans Slo-1 Loss of
631 Function Mutants.” *PLoS Pathogens*. https://doi.org/10.1371/journal.ppat.1001330.
632 Willson, James, Kiran Amliwala, Andrew Davis, Alan Cook, Matthew F. Cuttle, Neline Kriek,
633 Neil A. Hopper, et al. 2004. “Latrotoxin Receptor Signaling Engages the UNC-13-
634 Dependent Vesicle-Priming Pathway in C. Elegans.” *Current Biology*.
635 https://doi.org/10.1016/j.cub.2004.07.056.
636 Willson, J., K. Amliwala, A. Harder, L. Holden-Dye, and R. J. Walker. 2003. “The Effect of
637 the Anthelmintic Emodepside at the Neuromuscular Junction of the Parasitic Nematode
638 Ascaris Suum.” *Parasitology*. https://doi.org/10.1017/s0031182002002639.
639 Willson J, Holden-Dye L, Harder A, Walker RJ. 2001. “A Possible Mechanism for the Action
640 of the Novel Anthelmintic Emodepside, Using Ascaris Suum Body Wall Muscle
641 Preparations.” *The Journal of Physiology* 536 ((S103)): 132P – 133P.
642 Wit, Janneke, Clayton Dilks, and Erik Andersen. 2020. “Complementary Approaches to
643 Understand Anthelmintic Resistance Using Free-Living and Parasitic Nematodes.”
644 *Trends in Parasitology*. https://doi.org/10.20944/preprints202008.0313.v1.
645 Zahner, H., A. Taubert, A. Harder, and G. von Samson-Himmelstjerna. 2001. “Filaricidal
646 Efficacy of Anthelmintically Active Cyclodepsipeptides.” *International Journal for*
647 *Parasitology* 31 (13): 1515–22.
648 Zamanian, Mostafa, Daniel E. Cook, Stefan Zdraljevic, Shannon C. Brady, Daehan Lee,
649 Junho Lee, and Erik C. Andersen. 2018. “Discovery of Genomic Intervals That Underlie
650 Nematode Responses to Benzimidazoles.” *PLoS Neglected Tropical Diseases* 12 (3):
651 e0006368.
652 Zdraljevic, Stefan, Bennett William Fox, Christine Strand, Oishika Panda, Francisco J. Tenjo,
653 Shannon C. Brady, Tim A. Crombie, John G. Doench, Frank C. Schroeder, and Erik C.
654 Andersen. 2019. “Natural Variation in C. Elegans Arsenic Toxicity Is Explained by
655 Differences in Branched Chain Amino Acid Metabolism.” *eLife* 8 (April).
656 https://doi.org/10.7554/eLife.40260.
657 Zdraljevic, Stefan, Christine Strand, Hannah S. Seidel, Daniel E. Cook, John G. Doench, and
658 Erik C. Andersen. 2017. “Natural Variation in a Single Amino Acid Substitution Underlies
659 Physiological Responses to Topoisomerase II Poisons.” *PLoS Genetics* 13 (7):
660 e1006891.