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2 Opposing directions of stage-specific body length change in a close relative of *C. elegans*

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Abstract

Background

Body size is a fundamental organismal trait. However, as body size and ecological contexts
change across developmental time, evolutionary divergence may cause unexpected patterns of
body size diversity among developmental stages. This may be particularly evident in polyphenic
developmental stages specialized for dispersal. The dauer larva is such a stage in nematodes, and
Caenorhabditis species disperse by traveling on invertebrate carriers. Here, we describe the
morphology of the dispersal dauer larva of the nematode *Caenorhabditis inopinata*, whose adults
can grow to be nearly twice as long as its close relative, the model organism *C. elegans*.

Results

We find that the *C. inopinata* dauer larva is shorter and fatter than those of its close relatives *C.
elegans*, *C. briggsae*, and *C. tropicalis*, despite its much longer adult stage. Additionally, many
C. inopinata dauer larvae were ensheathed, an apparent novelty in this lineage reminiscent of the
infective juveniles of parasitic nematodes. We also found abundant variation in dauer formation
frequency among twenty-four wild isolates of *C. inopinata*, with many strains unable to produce
dauer larvae under laboratory conditions.

Conclusion

Most *Caenorhabditis* species thrive on rotting plants and disperse on snails, slugs, or isopods
(among others) whereas *C. inopinata* is ecologically divergent and thrives in fresh *Ficus septica*
figs and disperses on their pollinating wasps. These wasps are at least an order of magnitude
smaller in length than the vectors of other *Caenorhabditis* species. While there is some unknown
factor of the fig environment that promotes elongated body size in *C. inopinata* adults, the
smaller size of its fig wasp carrier may be driving the reduced body length of its dauer larva.
Thus ecological divergence across multiple developmental stages can promote unexpected and
opposing changes in body size within a single species.

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Background

61   One obvious fact of the life cycles of most organisms is that they typically get larger as they
62   develop. Indeed, most animals span at least an order of magnitude in body size within a single
63   generation through the course of development [1, 2]. However, a frequently neglected
64   consequence of this is that the ecological niche of an organism can change drastically within a
65   single individual throughout development. Notable examples of such size-structured ecological
66   niches (i.e., ontogenetic niches [3]) include animals exhibiting metamorphosis in development.
67   Ceratophryidae frogs have larval forms that eat small crustaceans and diatoms yet grow into
68   adult forms that eat larger arthropods, gastropods, and small vertebrates [4]. Silkworms eat
69   mulberry leaves as larvae yet develop into short-lived, non-feeding adults specialized for
70   reproduction [5, 6]. Such stage-specific ecology is not restricted to animals with dramatic
71   metamorphic development. Mammals usually begin postembryonic life consuming milk before
72   maturing into adults that consume plants, other animals, or both [1]. Many species of fish also
73   exhibit stage-specific resource use, and largemouth bass eats planktonic crustaceans, crawfish,
74   and cyprinid fish as it grows [3, 7].

75   The ontogenetic niche of an organism is not limited by resource use. Organisms often have
76   specialized developmental stages for specific life history strategies, and such partitioning is
77   frequently used for dispersal. Locusts have a complex polyphenism in overcrowding conditions
78   that transform solitary morphs into gregarious morphs that constitute famine-inducing swarms
79   [8]. Aphids also have a polyphenism specialized for dispersal where winged morphs arise in
80   harsh conditions; dimorphic aphids also are easily distinguished by thorax size differences [9].
81   The seeds of plants and spores of fungi are small stress-resistant propagules that frequently
82   harbor traits that aid in dispersal such as wings or barbs (in the case of seeds) [10, 11]. Thus life
83   history strategies can also influence stage-specific traits including size.

84   As adults, the free-living bacterivorous nematodes *Caenorhabditis elegans* and *C. inopinata* live
85   in substantially different habitats and differ substantially in size. In crowded, low food, high
86   temperature, or otherwise stressful conditions, *C. elegans* is known to develop into a dispersal-
87   specialized juvenile phase known as the dauer larva [12]. The dauer larva is a long-lived,
88   desiccation-resistant, and stress-resistant dispersal stage. In its natural context, *C. elegans* dauer
89   larvae travel to new resources on invertebrate carriers such as snails, slugs, isopods, and
90   myriapods [13]. The dauer exhibits a stage-specific behavior in nictation, wherein the animal
91   climbs a substrate and waves its head in the air to promote invertebrate-mediated dispersal [14].
92   Once it has traveled to a new rotting plant resource patch, the dauer larva will disembark and
93   directly develop into a reproductive stage animal [13]. As the dauer larva is a specialized L3
94   stage, it has a distinct morphology from reproductive phase animals [12]. While a great deal is
95   known about the *C. elegans* dauer stage, the dauer stage from *C. inopinata* has never before been
96   described. Do the substantial differences observed in the adult species of these close relatives
97   persist throughout this developmental stage as well?

98   Here, we describe the dauer larva of *Caenorhabditis inopinata*. While *C. inopinata* adults grow
99   twice as long and *C. elegans*, they also develop nearly twice as slowly as *C. elegans* [15-17].
100 Additionally, instead of thriving on rotting plants and dispersing on large invertebrates, *C.*

inopinata lives in fresh *Ficus septica* figs and disperses on fig wasp pollinators [15, 18]. Here we show that, rather than echoing the size differences observed in the adults, *C. inopinata* dauers are shorter and fatter than *C. elegans* dauers. In addition, *C. inopinata* dauers also exhibit a novelty in ensheathment that resembles the infective larvae of parasitic nematodes. Thus the evolution of body size need not be in the same direction across all developmental stages, and stage-specific ecological contexts can drive opposing directions of body size change.

Results

C. inopinata adults are much longer than those of *C. elegans* (Figure 1A; [15, 16]). However, as *C. inopinata* disperses on fig wasp vectors that are millimeters long, we were curious about the extent of body size change in its dispersal dauer larva. In *C. elegans*, dauers are typically isolated via SDS exposure [19], which kills non-dauer stages that can feed and lack a buccal plug. This approach has also been shown to isolate dauer larvae in *C. briggsae* [20]. We also successfully used this approach to isolate and characterize the morphology of *C. inopinata* dauer larvae (Fig 1. B-D). The vast majority of *C. inopinata* animals that survived SDS exposure had a characteristic buccal plug (Fig. 1C; 98%, 45/46 worms), revealing this is a sound method of recovering animals of the appropriate stage. Surprisingly, despite the elongation of *C. inopinata* adults compared to *C. elegans* (Fig. 1A), *C. inopinata* dauers are shorter (*C. inopinata*: mean = 430 microns, SD = 45 microns; *C. elegans*: mean= 456 microns, SD = 65 microns; Wilcoxon rank sum test $p = 0.0092$, W = 4337) and fatter (*C. inopinata*: mean = 27 microns, SD = 4.1 microns; *C. elegans*: mean= 18 microns, SD = 3.5 microns; Wilcoxon rank sum test $p < 2.2 \times 10^{-16}$, W = 10530) than its close relatives (Fig. 1-2). This leads to the dauer larva occupying different regions of morphological space in *C. elegans* and *C. inopinata* when compared with other developmental stages (Figure 2A). And while *C. elegans*, *C. briggsae*, and *C. tropicalis* differ in dauer length (Figure 2B; Tukey test $p = 1.4 \times 10^{-11}$), they have negligible differences in width (*C. tropicalis* is on average one micron wider than *C. elegans*; Tukey test $p = 0.020$), revealing the *C. inopinata* dauer to be an outlier with a length:width ratio that is 40% smaller than its close relatives (Figure 2B). Thus *C. inopinata* has a divergent dauer stage morphology that is surprisingly inconsistent with its elongated adult stage. Additionally, a notable fraction of *C. inopinata* dauer larvae retained their cuticle from the previous molt (46%, 21/46 worms). Reminiscent of the ensheathed infective larvae of parasitic nematodes [21, 22], this was never observed in *C. elegans* dauers (n=47 worms).

Anecdotally, it was clear that we were recovering low numbers of *C. inopinata* dauers in our SDS treatments. As variation in dauer formation frequency has long been noted in *C. elegans* [23-25], we attempted to isolate dauers from a number of *C. inopinata* wild strains from Okinawan *Ficus septica* figs (Figure 3; Supplemental Table Sheets 1-2; Supplemental Figure 1). As observed in *C. elegans*, there is variation in dauer formation frequency in *C. inopinata* among 29 lines (Figure 3; range = 0-2%, mean = 0.2%, sd= 0.4). However, most lines generate dauers at a low frequency, and many lines never produced dauers (11 lines; Figure 3). Furthermore, there appears to be no relationship between island of origin and dauer formation frequency (Figure 3; Wilcoxon rank sum test $p = 0.47$, W = 4068). This is in contrast to other *Caenorhabditis* species, where many lines are able to produce abundant dauers in starvation conditions (Supplemental Figure 2, [20, 23-25]). Thus the ecological divergence of *C. inopinata* may have impacted the dauer entry and exit decisions in this species, leading to its low propensity to promote dauer formation in laboratory conditions.
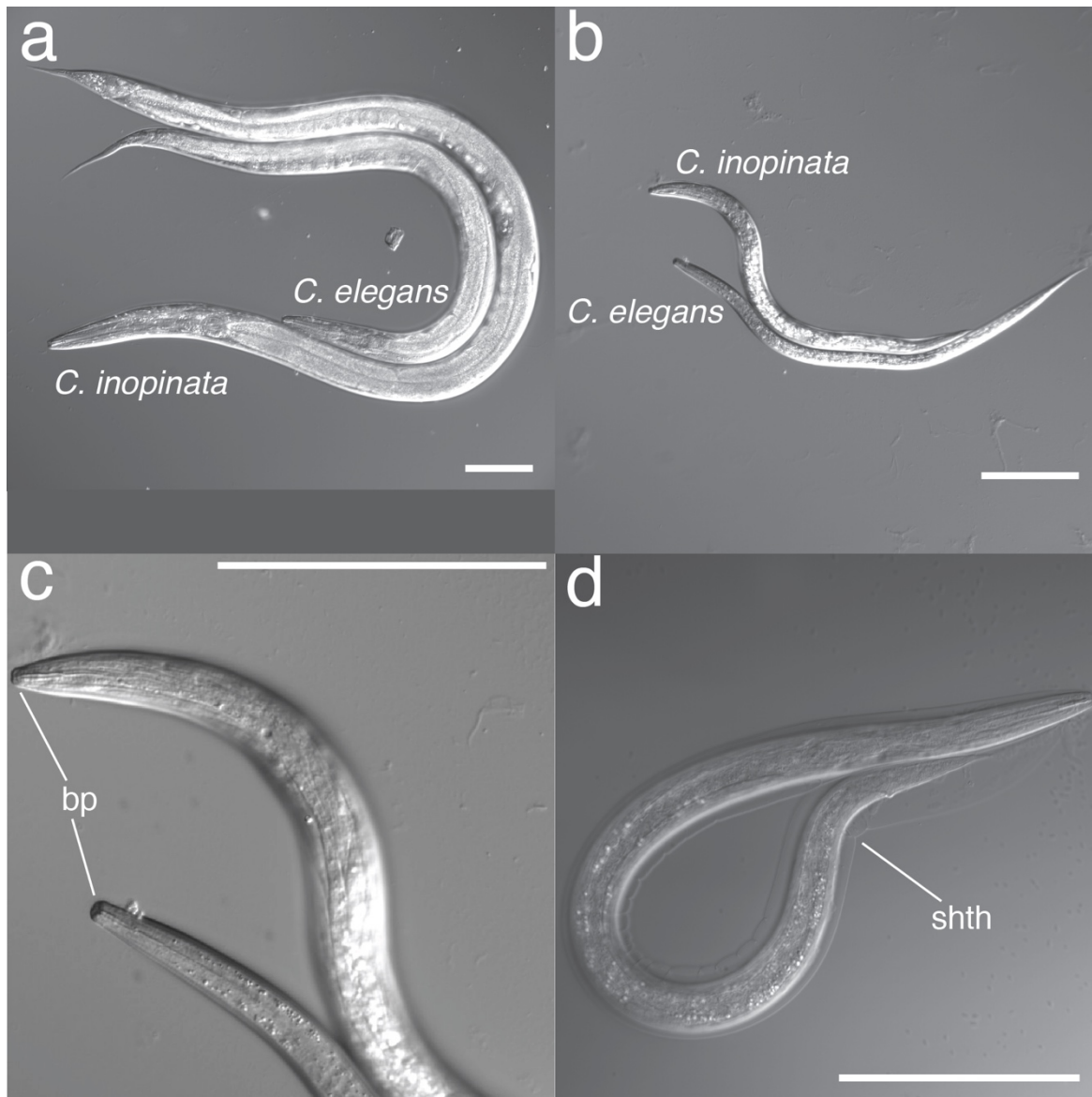
Figure 1. The *C. inopinata* dauer is morphologically distinct. (a) *C. elegans* adult hermaphrodite with *C. inopinata* adult female. (b) *C. elegans* and *C. inopinata* dauer larvae. (c) Enlargement of panel (b) showing the buccal plug. Bp, buccal plug. (d) An ensheathed *C. inopinata* dauer larva. Shth, sheath. All scale bars represent 100 microns.

Discussion

The partitioning of life history strategies among developmental stages is widespread. Among the most speciose groups of animals, holometabolous insects have just this kind of life cycle: larvae are specialized for growth while adults are suited for reproduction and dispersal [26]. This sorting leads to dramatic morphological and ecological differentiation among stages, with adults being nearly unrecognizable compared to their younger forms. Thus the allotment of such
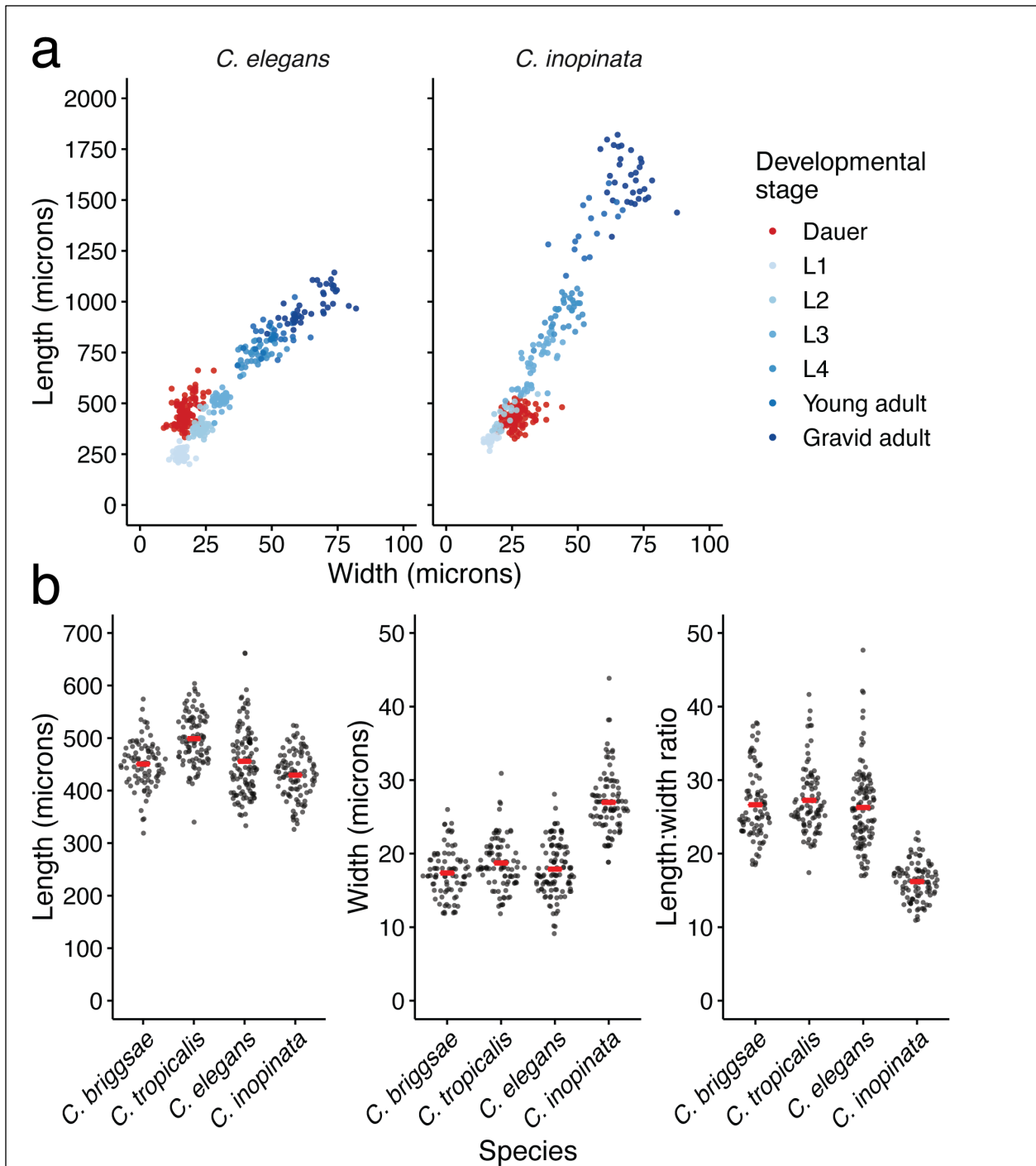
Figure 2. Quantification of dauer size. (a) Scatterplots revealing the length and width of various developmental stages. Data for non-dauer stages are from Woodruff et al. 2018. Left panel, *C. elegans*; Right panel, *C. inopinata*. Non-dauer stages N_{worms}= 16-103; *C. elegans* dauers, N_{worms}= 165; *C. inopinata* dauers, N_{worms}= 132. (b) Sina plots (strip charts with points taking the contours of a violin plot) illustrating the distributions of dauer length (left panel), dauer width (middle panel), and the dauer length:width ratio (right panel) of four *Caenorthabditis* species. Each dot represents the observation of one worm. Red bars represent averages. Sina plots take *C. elegans*, N_{worms} = 113; *C. inopinata*, N_{worms} = 97; *C. briggsae*, N_{worms} = 85; *C. tropicalis*, N_{worms} = 93.

Figure 3. Variation in dauer formation frequency in *C. inopinata.* Twenty-five *C. inopinata* strains are shown on the x-axis; all are wild isolates with the exception of NKZ43, which is a twenty-generation inbred line derived from NKZ2. Each point represents one observation of dauer formation frequency, the fraction of a starved population of animals surviving SDS treatment (N observations per strain = 2-10 ; N worms per observation = 17- 1557, average = 450, median = 397). Black horizontal lines represent the average. Strains are colored by Okinawan island of origin (Supplemental_tables.xls Sheet 1).
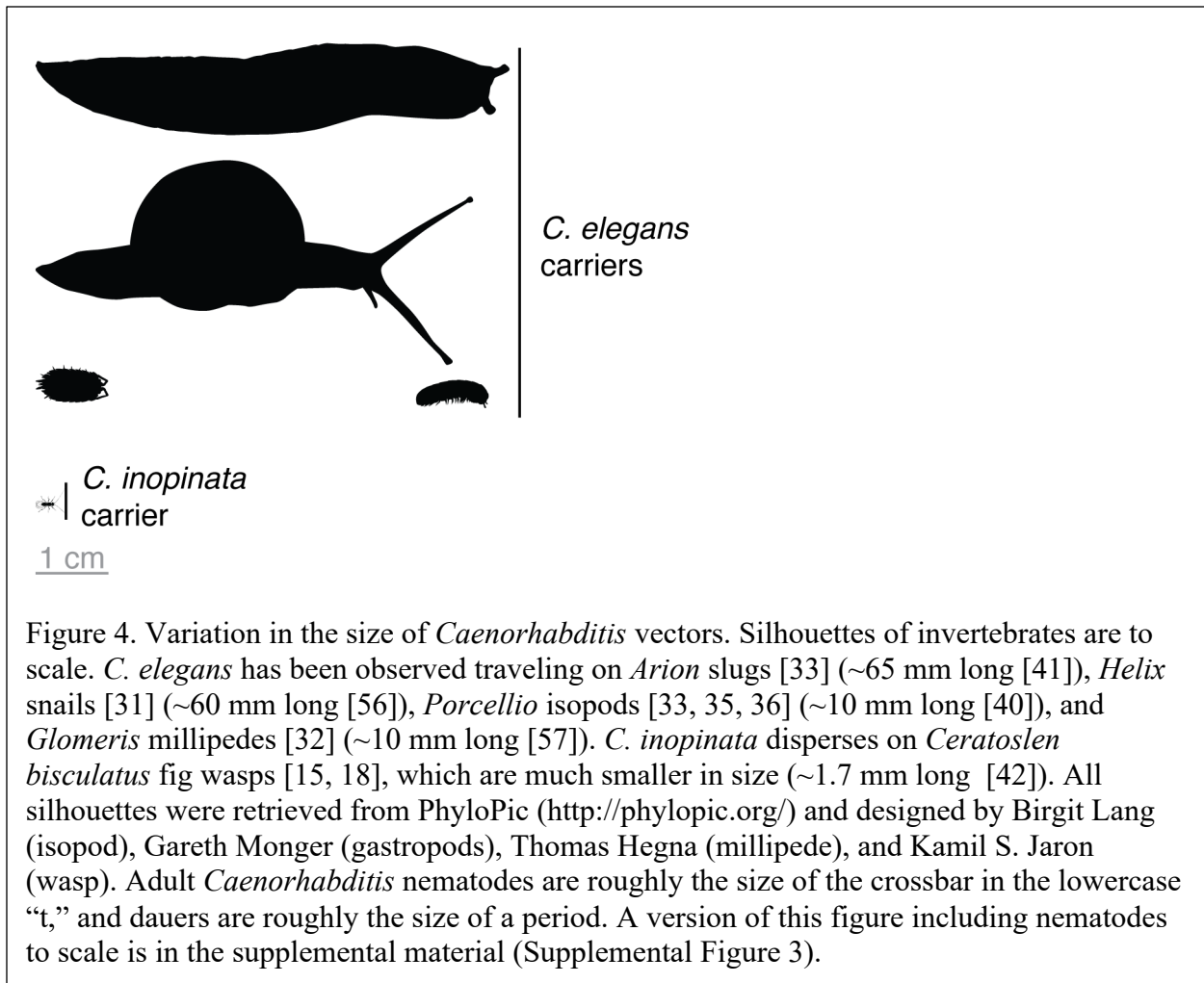
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154 strategies can drive marked diversity across development within a single organism. And although
155 many animals do not undergo striking metamorphosis throughout their development, diapause
156 and dispersal are pervasive approaches for responding to environmental change and for
157 promoting reproductive assurance [27]. These are frequently tied to specialized stages and often
158 require phenotypic plasticity to mediate entrance and exit from such forms at the appropriate
159 time [28]. And as this is usually accompanied by morphological and physiological adaptations
160 suited for these strategies, the partitioning of life history strategies across development provides
161 ample opportunity for the generation of variation both within and between individuals.

162 The nematode dauer larva represents such a stage: it is a stress-resistant, long-lived alternative
163 developmental stage specialized for dispersal whose development is induced by adverse
164 environments. Here, we described the broad morphological characteristics of the *C. inopinata*
165 dauer larva and found that it is shorter and fatter than the dauers of other *Caenorhabditis* species,
166 including *C. elegans* (Fig. 1-2). This finding is unexpected as *C. inopinata* is easily distinguished
167 from its close relatives by its exceptionally elongated adult body size [15, 16]. Indeed, *C.*
168 *inopinata* has been reported to grow as much as nearly twice [16] to three times [15] longer than
169 *C. elegans*. Thus its comparatively shorter dauer larva is seemingly incongruent with its longer
170 adult form. However, this observation can be clarified by situating *Caenorhabditis* dauer larvae
171 in their ecological contexts.

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172 Most *Caenorhabditis* species thrive by consuming the microbes in rotting plant material such as
173 stems, fruit, and flowers [13, 29]. In favorable environmental conditions, animals directly
174 develop into reproductively mature adults. In poor conditions, animals develop into the
175 alternative dauer larval stage [12]. Dauer larvae harbor a number of stage-specific traits that
176 promote dispersal including nictation, wherein animals climb a substrate and wave their heads in
177 the air [14], and experimental evidence suggests this behavior aids in phoresis on invertebrate
178 carriers [30]. *Caenorhabditis* dauer larvae have been frequently observed on such vectors, and
179 they presumably carry dauers to new rotting plant resources where they disembark and mature.
180 *C. elegans* has been observed on snails (*Helix*, *Oxychilus*, and *Pomatias* species [31, 32]), slugs
181 (*Deroceras*, *Arion*, and *Ambigolimax* species [31, 33, 34]), isopods (*Porcellio*, *Oniscus*, and
182 *Armadillidium* species [33, 35, 36]), millipedes (*Glomeris* species [32]), and centipedes [33, 37].
183 *C. briggsae* and *C. remanei* have also been observed on similar vectors (gastropods, isopods, and
184 myriapods [13, 34, 37-39]. Thus some *Caenorhabditis* species can disperse on a diverse array of
185 invertebrate carriers than span ~10-65 mm in body length (Fig. 4; [40, 41]). *C. inopinata*, on the
186 other hand, thrives in an entirely different ecological context, the lumen of fresh *Ficus septica*
187 figs [15, 18]. Furthermore, instead of dispersing on a variety of invertebrate carriers, *C.*
188 *inopinata* primarily travels on *Ceratosolen* pollinating fig wasps [15, 18]. Although *C. inopinata*
189 has been observed on the parasitic fig wasp *Phylotrypesis* [15], it appears to preferentially
190 embark on *Ceratosolen* pollinators [15, 18]. Thus *C. inopinata* is notable in its degree of host
191 vector specialization. Furthermore, fig wasp vectors are up to two orders of magnitude smaller in
192 length than the vectors of *C. elegans* and other rotting plant-associated *Caenorhabditis* species
193 (Fig. 4; [42]). Thus, although there must be some (as yet unknown) factor of the fig environment
194 driving increased adult body length in *C. inopinata* adults, the divergent morphology of its dauer
195 larvae can likely be at least partly explained by its need to disperse on much smaller vectors (Fig.
196 4). In this way, the divergent ecological contexts of different developmental stages can drive
197 morphological divergence of such stages in opposing directions.

198 Such ecological divergence in host vectors may also explain the novel ensheathment trait
199 observed in *C. inopinata* dauer larvae (Fig. 1D). The retention of the L2 cuticle in diapause
200 larvae, or ensheathment, is widespread in nematodes [21]. Often associated with the infective
201 larvae of parasitic species, ensheathment is thought to aid in desiccation resistance [43],
202 predation resistance [44], and freezing tolerance [45]. Furthermore, in many parasitic nematodes,
203 passage through an ensheathed infective (i.e., dauer) larval stage is essential for progression
204 through the life cycle [21, 46]. At the same time, it is important to note that ensheathment is not
205 restricted to parasites and has been observed in free-living nematodes [47]. To the best of our
206 knowledge, ensheathment has never been observed before in *Caenorhabditis* nematodes. This
207 includes the vector specialist *C. drosophilae*, whose dauer larva is also associated with a
208 comparatively small host (*Drosophila nigirospiracula*; thorax length ~1.3 mm [48]) but is not
209 ensheathed [49]. Thus it is possible that vector size alone is not driving the evolution of this trait
210 in *C. inopinata*. Dissections of *Ceratosolen* fig wasps have revealed the presence of *C. inopinata*
211 dauer-like larvae in wasp hemolymph [15]; thus ensheathment may be important for dauer
212 survival in this novel environment. However, presumably unsheathed *Caenorhabditis* species
213 have been observed in the guts of snails and slugs [33, 38], so ensheathment is possibly not
214 needed for the survival of internal passage in invertebrate carriers *per se*. Additionally, although
215 *C. inopinata*'s relationship with fig wasps is thought to be phoretic (like with other
216 *Caenorhabditis* species and their invertebrate carriers), it remains possible that they negatively
217 impact fig wasp fitness. Although direct fig wasp parasitism seems unlikely as *C. inopinata* is

Figure 4. Variation in the size of *Caenorhabditis* vectors. Silhouettes of invertebrates are to scale. *C. elegans* has been observed traveling on *Arion* slugs [33] (~65 mm long [41]), *Helix* snails [31] (~60 mm long [56]), *Porcellio* isopods [33, 35, 36] (~10 mm long [40]), and *Glomeris* millipedes [32] (~10 mm long [57]). *C. inopinata* disperses on *Ceratoslen bisculatus* fig wasps [15, 18], which are much smaller in size (~1.7 mm long [42]). All silhouettes were retrieved from PhyloPic (http://phylopic.org/) and designed by Birgit Lang (isopod), Gareth Monger (gastropods), Thomas Hegna (millipede), and Kamil S. Jaron (wasp). Adult *Caenorhabditis* nematodes are roughly the size of the crossbar in the lowercase "t," and dauers are roughly the size of a period. A version of this figure including nematodes to scale is in the supplemental material (Supplemental Figure 3).

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219 bacterivorous and culturable in the lab without fig wasps [15-17], *C. inopinata* may be
220 necromenic or entomopathogenic. Entomopathogenic *Steinernema* species have ensheathed
221 larvae and carry the pathogenic bacteria that kills their slug hosts and allows them to thrive [50].
222 Furthermore, the dauer larvae of fig wasp-associated *Parasitodiplogaster* species have also been
223 observed to be ensheathed [51]. This could be further evidence that ensheathment may be needed
224 for successful transit on fig wasps in particular. However, fig-associated *Schistonchus* nematodes
225 often do not have apparent dauer larvae (with or without ensheathment) as the infective stage is
226 usually the pre-reproductive female [52]. Regardless, as *C. inopinata* has gained a trait
227 commonly associated with animal parasitism, this affords the opportunity of using the
228 *Caenorhabditis* model system genetics toolkit to potentially understand the evolution of
229 parasitism-related traits.

230 Variation in dauer formation frequency was also observed in *C. inopinata* (Fig. 3). In *C. elegans*,
231 there is substantial variation in this trait [23-25], and it has a complex genetic basis, with at least
232 thirty-six loci contributing to most of the variance in dauer formation frequency [25].
233 Additionally, most isolates produced small numbers of dauer larvae, with many producing none
234 at all (Fig. 3). As it is likely that fig and/or fig wasp cues influence the dauer decision in natural
235 conditions [18], it is possible that their absence in laboratory culture promotes the low dauer
236 formation frequencies observed in *C. inopinata*. Future studies will be needed to disentangle the

237 roles of dauer entry, exit, and their interactions with figs and fig wasps in the variation of this
238 trait. Furthermore, we observed no clear relationship between island of origin and dauer
239 formation frequency in these lines; this is consistent with preliminary population genomic data
240 that reveal little genetic differentiation between Okinawan island populations (Woodruff et al.,
241 manuscript in prep.). Regardless, future quantitative genetic studies using these isolates will
242 prove invaluable for understanding the genetic basis of dauer formation and other quantitative
243 traits in *C. inopinata*.

244 **Conclusions**

245 Here, we showed that the *C. inopinata* dauer larva has decreased length despite its elongated
246 adult body size compared to *C. elegans*. This is likely due to the divergent ecological contexts of
247 these developmental stages that are specialized for dispersal or reproduction. Fig wasps are the
248 dispersal vector of *C. inopinata* that is at least an order of magnitude smaller than those used by
249 *C. elegans*, while figs have potentially released some selective constraint that allows larger adult
250 body sizes compared to the rotting plant environments of *C. elegans*. Thus polyphenisms and
251 specialized developmental stages reveal surprising flexibility in the direction and magnitude of
252 body size evolution in a single species.

253 **Methods**

254 *Strains and maintenance*

255 Animals were grown on modified Nematode Growth Media with *Escherichia coli* strain OP50-1
256 for food as in [17]. All animals were raised at 25°C. Strains *C. inopinata* NKZ2 (also known as
257 NK74SC [15, 16]), *C. elegans* N2, *C. briggsae* HK104, and *C. tropicalis* NIC122 were used for
258 dauer morphology observations. For characterizing variation in dauer formation frequency,
259 multiple strains of *C. elegans* (ED3040, CB4856, JU1088, JU775, MY16), *C. briggsae* (JU1348,
260 ED3091, HK104), and *C. tropicalis* (NIC122, QG131) were used.

261 We report multiple new wild isolates of *C. inopinata* used for characterizing dauer formation
262 variation. *C. inopinata* strains NKZ22 and NKZ27 were isolated from fresh *F. septica* figs from
263 the island of Iriomote, Okinawa, Japan in May 2014 (Supplemental Figure 1;
264 supplemental_tables.xls, Sheet 1). *C. inopinata* strains NKZ44, NKZ45, NKZ46, NKZ47,
265 NKZ49, NKZ50, NKZ51, NKZ52, NKZ54, NKZ55, NKZ56, NKZ57, NKZ59, NKZ60, NKZ63,
266 NKZ64, NKZ66, NKZ67, NKZ68, NKZ69, NKZ70, NKZ72, NKZ73, NKZ75, and NKZ88 were
267 isolated from fresh *F. septica* figs from the islands of Iriomote and Ishigaki, Okinawa, Japan in
268 May 2015 (Supplemental Figure 1; supplemental_tables.xls, Sheet 1). *C. inopinata* strains
269 PX723 and PX724 were isolated from fresh *F. septica* figs from Taipei, Taiwan in August 2019
270 (Supplemental Figure 1; supplemental_tables.xls, Sheet 1). Wild isolates were established as in
271 [16]. Briefly, figs were placed in a petri dish filled with water or M9 buffer. Figs were then cut
272 into four pieces; worms subsequently found in suspension were placed on to NGM plates to
273 establish wild isolates. Morphology and association with *F. septica* strongly suggest these wild
274 isolates are the same species as *C. inopinata*. Molecular barcoding with the ITS2 (ribosomal
275 rDNA internal transcribed spacer-2) sequence and/or mating tests were performed in a few of
276 these lines, confirming their species identity (supplemental_tables.xls, Sheet 2; [53]). As mating
277 tests have not been performed in a number of lines, there exists the unlikely possibility these

278 particular lines are cryptic species distinct from *C. inopinata*. *C. inopinata* strain NKZ43 is an
279 inbred line of *C. inopinata* made through 20 generations of sib-pair inbreeding (derived from the
280 genome-sequenced strain NKZ35 [15]).

281 *Dauer isolation*

282 Dauer larvae were isolated 10-14 day old starved cultures incubated at 25°C. Animals were
283 washed off of plates in M9 buffer and then incubated in 1% SDS for a half hour. Animals were
284 then washed four times in M9 buffer and plated. Live worms were then used for subsequent
285 observations.

286 *Microscopy and measurements*

287 Animals were mounted on agar pads and imaged on a dissecting microscope. Animal length and
288 width was measured using the ImageJ software [54]; curved lines were addressed with the
289 "segmented line tool" as in [55]). All measures of reproductive, non-dauer stages were retrieved
290 from the data in [16]. The fraction of animals surviving SDS treatment was determined by
291 counting the number of live and dead worms from a sample of a given suspension both before
292 and after SDS exposure. All statistics were performed in the R language, and all data and code
293 associated with this study have been deposited on Github
294 (https://github.com/gcwoodruff/dauer_2020).

295 **List of abbreviations**

296 L3, third larval stage. SDS, Sodium dodecyl sulfate.

297 **Declarations**

298 *Ethics approval and consent to participate*

299 Not applicable.

300 *Consent for publication*

301 Not applicable.

302 *Availability of data and material*

303 All data and code not included as Additional Files have been posted to Github
304 (https://github.com/gcwoodruff/dauer_2020).

305 *Competing interests*

306 The authors declare that they have no competing interests.

307 *Funding*

11

310 *Authors' contributions*

311 GCW, PCP, and EH designed the experiments; EH and GCW performed the experiments; EH
312 and GCW analyzed the results; EH, GCW, and PCP wrote the paper.

313 *Acknowledgements*

316 **Additional Files**

317 Additional File 1. Supplemental Figures (Supplemental_figures.doc).

318 Additional File 2. Supplemental Tables (Supplemental_tables.xls).

319 **Supplemental Figures**

320 Supplemental Figure 1. Localities of *C. inopinata* strains isolated from Okinawa (islands of
321 Iriomote and Ishigaki) and Taiwan.

322 Supplemental Figure 2. *C. inopinata* produces few dauer larvae compared to its close relatives.

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