NEUROSCIENCE

Sex or cannibalism: Polyphenism and kin recognition control social action strategies in nematodes

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Resource polyphenisms, where single genotypes produce alternative feeding strategies in response to changing environments, are thought to be facilitators of evolutionary novelty. However, understanding the interplay between environment, morphology, and behavior and its significance is complex. We explore a radiation of *Pristionchus* nematodes with discrete polyphenic mouth forms and associated microbivorous versus cannibalistic traits. Notably, comparing 29 *Pristionchus* species reveals that reproductive mode strongly correlates with mouth-form plasticity. Male-female species exhibit the microbivorous morph and avoid parent-offspring conflict as indicated by genetic hybrids. In contrast, hermaphroditic species display cannibalistic morphs encouraging competition. Testing predation between 36 co-occurring strains of the hermaphrodite *P. pacificus* showed that killing inversely correlates with genomic relatedness. These empirical data together with theory reveal that polyphenism (plasticity), kin recognition, and relatedness are three major factors that shape cannibalistic behaviors. Thus, developmental plasticity influences cooperative versus competitive social action strategies in diverse animals.

INTRODUCTION

Resource polyphenisms are plastic traits facilitating the exploitation of alternative resources in response to environmental pressures on an organism. These polyphenic traits can have a profound influence on an organism's morphology, ecology, physiology, and behavior, and they have been observed among diverse taxa (1–4). Examples of resource polyphenisms can be seen in alternative feeding morphs, where distinct feeding structures and, subsequently, different feeding strategies are found between discrete morphs. One of the most dramatic examples of alternate feeding strategies are predatory versus nonpredatory and even cannibalistic versus noncannibalistic forms, resulting in diverse diets and the induction of aggressive behaviors (5–9). Furthermore, some cannibal morphs display recognition of self and relatives, therefore reducing the risk of harming progeny and kin (5–10). However, understanding the complex network of influences acting on the developmental decision behind cannibalistic versus noncannibalistic polyphenisms is difficult, as it requires a combination of ecological insights together with genetic, molecular, and, frequently, behavioral analysis, which is not readily available in many species.

In diplogastrid nematodes of the genus *Pristionchus*, a polyphenic trait exists in which one of two alternative mouth morphs develop. This irreversible developmental decision results in the formation of either the *stenostomatous* (St) morph, where animals have a single tooth with a narrow mouth cavity, or, alternatively, the *eurystomatous* (Eu) morph, whereby the animal is wide mouthed with two teeth (Fig. 1A) (11, 12). Coinciding with these morphological distinctions, behavioral differences between these morphs are evident: While the St mouth form feeds on bacteria, the Eu morph is omnivorous and

capable of supplementing its bacterial diet by predating on the larvae of other nematode species, including feeding on conspecifics (Fig. 1B) (13). In addition, this predatory biting behavior also serves to repel potential competitors from their location, as while adult nematode cuticles are sufficient to prevent penetration and death, attacks do provoke an avoidance behavior in the recipient, which can cause their dispersal from the limited nutrient sources (14). The formation of the Eu morph is associated with an adaptive cost in the form of an increase in developmental time (15, 16). In one species, *P. pacificus*, a wide range of molecular and genetic tools are available (17–20). As such, the mouth-form decision and the associated predatory behaviors have been extensively studied and are dependent on genetic and environmental factors (21–27). Furthermore, alongside the predatory behaviors, a self-recognition system exists based on the small peptide signal *self-1*, which promotes the killing and cannibalism of progeny of intraspecific competitors but not of self-progeny (28). We investigated a radiation of 29 *Pristionchus* species and unexpectedly found that mouth-form preference shows a strong correlation with reproductive mode. Empirical and theoretical evidence indicates the interaction of several major factors—plasticity, kin recognition, and relatedness—that determine the most appropriate social action strategy in individual species.

RESULTS

Mouth form correlates with reproductive mode

Previous studies on mouth-form plasticity and predation in *Pristionchus* have focused nearly exclusively on the hermaphrodite *P. pacificus*; however, this organism is only one species in a well-described phylogeny thus far encompassing around 50 species (29). Furthermore, within the genus *Pristionchus*, two different modes of reproduction are observed, gonochorism and hermaphroditism. The ancestral gonochoristic reproductive mode is most common and requires obligate mating between females and males. In contrast, hermaphroditic species are capable of self-fertilizing and propagating without a mating partner; however, this severely limits their genetic diversity and results in highly related populations (30). In *Pristionchus*,
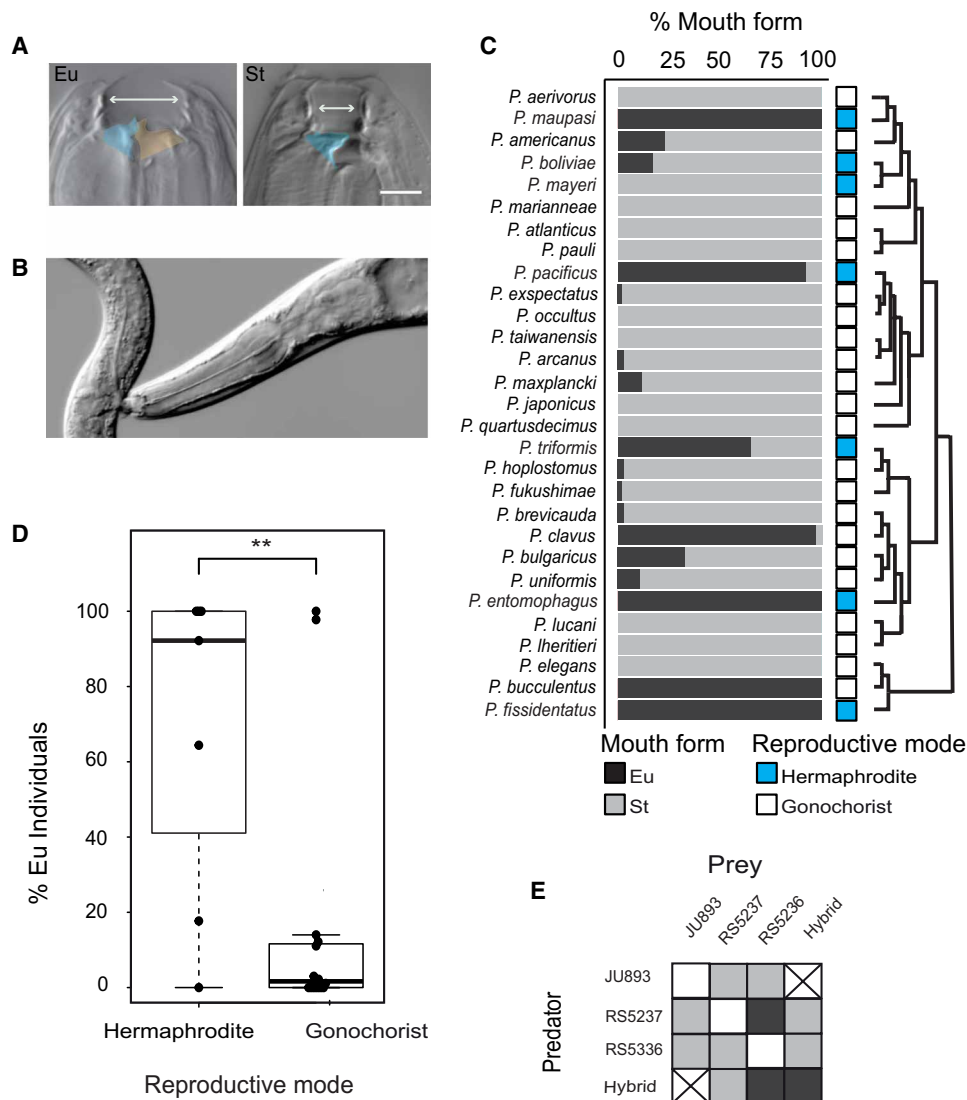
Fig. 1. Plasticity and reproductive mode correlate in *Pristionchus* nematodes. (**A**) *Pristionchus* mouth-form dimorphism. *P. pacificus* predatory eurystomatous form (Eu), which has a larger mouth opening and two teeth compared to the nonpredatory stenostomatous form (St), which has a narrow mouth opening and a single tooth. Scale bar, 5 μm. (**B**) Killing behavior with a *P. pacificus* predator killing a *C. elegans* larva. (**C**) % Eu mouth-form frequency in 29 distinct *Pristionchus* species including phylogeny and their associated reproductive modes. Comparisons were made between hermaphrodites and females from gonochoristic species. Mouth-form frequency is the mean value of three independent replicates, each consisting of 30 animals. Note that, in many species, no Eu animals were detected. (**D**) Groupings of all species sharing reproductive strategy with mouth form associated with reproductive mode. **P < 0.01, showing significant association between reproductive mode and mouth-form preference. (**E**) Predator-prey killing grid among diverse *P. uniformis* strains from the United States (RS5237), Germany (RS5236), and France (JU893), and a hybrid strain generated by mating RS5237 and RS5236. Assays were conducted using rare Eu *P. uniformis* predators. All strains display a within-strain self-recognition system and between-strain killing. Color intensity represents killing efficiency with high (>30 corpses) shown in black, medium (one to 30 corpses) in gray, or low (0 corpses) in white. Data are the mean of three standard corpse assays. Crossed boxes indicated not tested.

this mating type has evolved at least seven times independently with eight known hermaphroditic species (*29*, *31*). As mating between conspecific females and males is necessary in gonochoristic species, how the reproductive mode may influence the mouth-form decision and its associated predatory behaviors is currently unknown.

Therefore, we first analyzed the mouth-form abundance across the *Pristionchus* phylogenetic clade by investigating 29 different species (Fig. 1C and table S1). Notably, mouth-form preference shows a strong association with reproductive mode [$P = 0.0043$, analysis of variance (ANOVA)], while no phylogenetic signal was detected

($P = 0.637$ and 0.769; for C_{mean} and Moran's *I* index, respectively) (Fig. 1D). The majority of gonochoristic species adopt the nonpredatory St morph (20 of 22 tested species). In contrast, hermaphroditic species appear to be affiliated with the predatory Eu morph in five of seven analyzed species. This mouth-form association is robustly maintained under diverse environmental conditions including different temperatures and diets (fig. S1). In the gonochoristic Eu species (*P. bucculentus* and *P. clavus*), limited available strains make it difficult to confirm whether these mouth-form associations are consistent throughout these species, whereas in the hermaphroditic

St-associated species (*P. mayeri* and *P. boliviae*), all available strains are St. Thus, our observations support a vision of parallel evolution of mouth-form fate and reproductive mode that may imply an adaptive value for such associations (*32*). Therefore, reproductive mode represents a previously neglected factor that influences the expression of cannibalistic behaviors in *Pristionchus* nematodes.

Mouth form promotes gonochoristic mating

Why would the gonochoristic reproductive mode associate with the nonpredatory St morph? We reasoned that the St bias might prevent fatal cannibalistic encounters between genetically distinct individuals in which the opportunity to mate is paramount. To test this hypothesis, we selected *P. uniformis* in which a multitude of genetically diverse natural isolates are available. All 13 analyzed *P. uniformis* strains confirmed a consistently strong St mouth-form association (fig. S2 and table S2), and reciprocal mating experiments among three *P. uniformis* strains from the United States, France, and Germany produced viable and fertile F2 progeny, validating the potential for these strains to be mating partners (fig. S3, A and B).

To test whether the nonpredatory St mouth form will facilitate mating and prevent killing between conspecifics, we selected rare Eu animals from these three strains and analyzed killing interactions (fig. S4). No killing was detected when predators were tested against prey of the same strain, indicating robust self-recognition (Fig. 1E). In contrast, cannibalism was observed between conspecifics with predators from all strains killing prey of all other genotypes. Moreover, when cross progeny between two different strains were tested in a reciprocal predator-prey setup, it showed mutual killing (Fig. 1E and table S3). Specifically, both parental predators killed their hybrid prey progeny, and similarly, hybrid predators killed prey of both parental lines. Last, hybrid predators killed their siblings in predator-prey assays. Together, these results indicate that the adoption of the non-predatory St mouth form in gonochoristic species may be an adaptive strategy that avoids the killing of potential mating partners and therefore facilitates mating. In addition, full siblings can be quite divergent in gonochoristic species, and thus the adoption of the nonpredatory morph will guarantee not to consume your sisters or brothers. In summary, through the establishment of an St mouth form in gonochoristic nematodes, they circumvent cannibalistic tendencies, which may repel potential mates (*14*) and kill hybrid larvae and thus avoid parent-offspring conflict.

Predatory traits in hermaphrodites promote competition

Hermaphroditic species are frequently affiliated with the predatory Eu morph, the opposite of that observed in gonochoristic species. As hermaphrodites do not require a mating partner, we hypothesized that it may be advantageous to adopt a predatory strategy by killing rivals and removing competitors, an example of intraguild predation (*33*, *34*). This could be particularly relevant, as reproduction via selfing minimizes genetic diversity and maintains close genetic relatedness with their offspring, which are not cannibalized by parents due to a self-recognition system (*28*). However, little is known about the ecological relevance of predation and cannibalism, as previous studies in *P. pacificus* focused exclusively on geographically diverse strains. To investigate the ecological significance of cannibalism, we used *P. pacificus* isolates from the small island La Réunion in the Indian Ocean (Fig. 2A), which, despite its age of only 2 to 3 million years, harbors a huge diversity of genetic lineages (*35*). We used 36 strains from three island populations at Trois Bassins, Grand Etang (GE),

and Nez de Boeuf, which frequently co-occur with a specific beetle species (Fig. 2B and table S1). These 36 strains were randomly chosen from a previous genomic meta-analysis of 264 La Réunion–based strains, and some of them are extremely closely related as indicated by their nucleotide diversity of $\pi < 0.001$ (*36*). Furthermore, genome analysis confirmed that these strains propagate exclusively by selfing with no heterozygosity observed in their genomes. We first analyzed the mouth-form ratios in these wild isolates for evidence of a particular morph. In all but two of these strains, the predatory Eu mouth form was the prevalent morph, potentially promoting competition and killing between strains (Fig. 2, C to E).

To test this hypothesis, we assessed the potential for killing and cannibalism between naturally co-occurring isolates. We set up pairwise killing assays between all 12 strains from each location to explore ecologically relevant predatory interactions, which revealed three distinct behaviors (Fig. 2, F to H, and fig. S4). First, we observed mutual cannibalism whereby strains kill one another with varying degrees of efficiency (63.1%). Second, we found multiple examples of one-directional killing, i.e., strain RSC066 from GE cannibalizes the strains RSC033, RSA054, and RS5407 but is not cannibalized in return. In total, we found that 24.3% of the strains displayed one-directional killing. Last, we detected examples of reciprocal recognition with some strains avoiding predatory behaviors altogether (12.6%). Notably, differing degrees of cannibalism were also observed between strains. Specifically, some strains exhibit extreme predation with more than 400 successful killing events in standardized corpse assays, whereas other strains show infrequent killing (table S4). Together, these results indicate that, in the hermaphroditic *P. pacificus*, the Eu morph is strongly favored and promotes competition between most, though not all, ecologically relevant conspecifics.

Genomic relatedness mediates killing strategies

Consequently, we wanted to understand the mechanism(s) behind the killing decision. Two theoretical assumptions could explain the observed behaviors. A single gene or allele(s) thereof could allow the recognition of other carriers, preventing cannibalism and resulting in a so-called "green beard effect" (*37–41*). Alternatively, the overall relatedness of strains might influence cannibalistic behaviors as originally proposed in kin selection theory by Hamilton (*37*, *42*). To distinguish between these possibilities, we first assessed whether the previously identified component of the self-recognition signal *self-1* acts as a green beard. *self-1* encodes for a secreted small peptide and contains a hypervariable C-terminal domain in which single amino acid alterations result in killing (*28*). If cannibalism were solely dependent on *self-1*, the competitive interactions between the La-Réunion–based strains should strictly correlate with the SELF-1 sequence in the hypervariable domain. Therefore, we identified *self-1* from RNA sequencing (RNA-seq) data and generated profiles of their hyper-variable domains for the majority of the 36 strains (Fig. 2, F to H). At all three locations, some strains shared an identical SELF-1 hypervariable domain (29 of 198 pairwise comparisons). We observed killing between 20 of these 29 strains. Thus, *self-1* alone is insufficient to predict killing outcome and is not a green beard.

Next, we analyzed whether the overall genomic relatedness is a predictor of the killing versus cooperation decision among strains (Fig. 2, I to K). Genome-wide relatedness between pairs was determined from the number of single-nucleotide polymorphisms (SNPs) as identified from RNA-seq data and was scaled relative to the population average. In this analysis, positive values indicate that strain
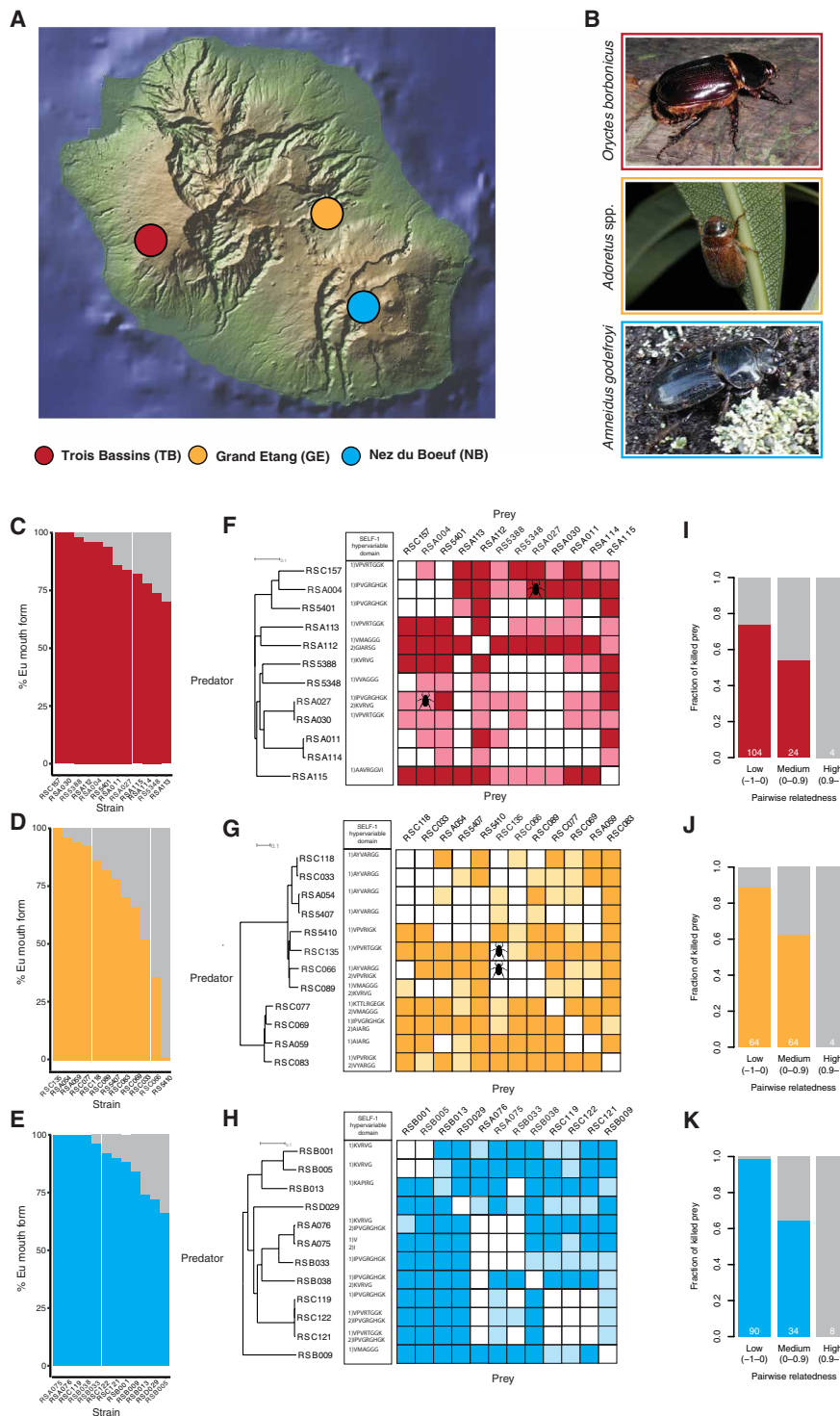
Fig. 2. Cannibalism is abundant in *P. pacificus*. (**A**) Strains from the hermaphroditic *P. pacificus*, isolated from three locations on La Réunion island. (**B**) *P. pacificus* from the three locations have distinct beetle associations. (**C** to **E**) Mouth-form decision is strongly biased toward the predatory Eu morph in the majority of wild isolates (*n* = 50 animals for each strain). (**F** to **H**) Predator-prey killing grids between strains at each location. Color intensity represents killing interactions resulting from the mean of two standard corpse assays, corresponding to either high (>30 corpses), medium (1 to 30 corpses), or low (0 corpses) killing frequencies. Strain phylogeny, SELF-1 copy number, and hypervariable sequences are also included. A beetle symbol represents pairs of strains isolated from the same beetle host. (**I** to **K**) Quantification of the fraction of killing interactions categorized according to the degree of relatedness between strains, calculated by the number of SNPs from RNA-seq data scaled relative to the population average. Numbers in each column indicate the total numbers of pairs in each category. The degree of relatedness between pairs is scored according to the thresholds, low (−1.0 to 0.0), medium (0.0 to 0.9), and high (0.9 to 1.0). Photo credit: Matthias Herrmann, MPI Developmental Biology, Germany; Jacques Rochat, Micropoda, France.

pairs are more closely related than the population average, while negative values indicate that pairs are more distantly related, as originally suggested by Hamilton's theoretical studies and their subsequent developments (see Materials and Methods) (*37*, *39*, *42*). We found that extremely closely related strains with an *r* value above 0.9 do not kill one another. In contrast, pairs of strains that are more distantly related than the population average are likely to kill each other (*P* < 0.05 across three populations, Fisher's exact test). Thus, strains, which are more closely related, frequently avoid cannibalistic behaviors, while more distantly related strains instead compete and kill one another. Furthermore, as killing correlates with the degree of genetic dissimilarity, this suggests multiple important genetic regions associated with nematode identity across the genome and demonstrates the ability of these nematodes to distinguish kin from non-kin with remarkable precision (Fig. 2, I to K). Thus, genomic relatedness informs the cannibalistic decision and overall interaction strategy.

Modeling cannibalistic strategies

Last, we modeled the costs and benefits of cannibalism and mouth-form plasticity in age-structured populations propagating by selfing, which represents the derived trait in *Pristionchus* evolution, by using two age classes of the Eu and St morphs, juveniles and adults (Fig. 3, A and B). Reproduction is biased toward one of the morphs. Adult population size is regulated by adult competition, and Eu adults can prey on all juveniles. When individuals of different strains *x* and *y* interact, predation depends on (i) population structure determined by the probability of encounter, (ii) their overall relatedness, and (iii) their ability for self-recognition (Fig. 3A). This approach allowed us to explore the circumstances that would result in the adoption of the Eu morph and its associated cannibalistic behavior.

First, we modeled under which conditions two strains would be able to coexist by modifying their degree of relatedness (*r*) and the encounter probability (*b*) (Fig. 3, C and D). Only under extremes of relatedness (*r* = 0.99 or 0.95) or in the absence of any encounter (*b* = 0) can *x* and *y* coexist for 100 steps. In contrast, if *x* and *y* are less related (*r* < 0.9), one strain will ultimately dominate. When strains are not interacting (*b* = 0), the St morph will surpass the Eu form, although both coexist. However, the Eu form will outcompete the St morph, with increased encounters and limited relatedness. These findings robustly corroborate our empirical data in the hermaphroditic *P. pacificus*. Second, a strong self-recognition system is required for the prevalence of the Eu morph and essential for population growth in general (fig. S5). Third, we used modeling to overcome the experimental limitations of the pairwise cannibalism assays. When modeling the interactions between three strains, we found that two highly related strains *x* and *z* more rapidly outcompeted a less related strain *y* (Fig. 3E). The coexistence of *x* and *z* may hint at a cooperative strategy between potential kin, allowing closely related strains to flourish while rapidly removing possible competitors. However, this initial "cooperation" will ultimately result in competitive interactions between *x* and *z*. Furthermore, the fixed genomic relatedness difference of 0.2 and 0.1 between strains *x* and *z* relative to *y* results in notably different population trajectories (Fig. 3E and fig. S6). These results are consistent with the empirical findings observed on La Réunion (Fig. 2). Together, therefore, modeling approaches indicate which conditions, in particular, overall genetic relatedness and self-recognition systems, are necessary to drive the prevalence of the predatory Eu morph and have likely facilitated the coexistence of cannibalistic forms.

DISCUSSION

Here, we integrate genome-level understanding of relatedness in a social action strategy and demonstrate through both empirical data and modeling that polyphenism (plasticity), kin recognition, and genomic relatedness shape cannibalistic behaviors. The unexpected association between mouth-form plasticity and reproductive mode during *Pristionchus* evolution suggests strong selection, resulting in the best action strategy to increase reproductive success (*39*). This includes the evolution of cooperative strategies in gonochoristic species while enhancing selfish actions in hermaphroditic ones. The correlation of phenotypically plastic traits and social interaction strategies may be a frequently observed principle, as it also has a role in inducing cooperative strategies in hymenopterans and termites (*43*) while also promoting competitive behaviors, as seen in salamanders, spadefoot toads, and rotifers (*5*, *6*, *9*, *10*). It is important to note that while the mouth-form fate observed in *Pristionchus* is consistently associated with reproductive mode, the ability to form the alternative mouth form is still maintained in most populations. Therefore, the alternative mouth form must be beneficial under certain environmental conditions and may be a bet-hedging strategy. Correspondingly, two gonochoristic and two hermaphroditic species show the opposite mouth-form association. It is possible that these species are in transition toward the canonically associated morph or these isolates are not representative of their species as a whole. Alternatively, they may be under different evolutionary pressures, maintaining the opposing mouth-form strategy, despite the accompanying impediments. As St animals still feed on the carcasses of other nematodes under laboratory conditions (*13*), a scavenging strategy is one potential system, which we have not yet investigated in the wild and which may facilitate the adoption of the alternative morph. Furthermore, a recent analysis of the decaying beetle environment on which many of these nematodes are associated revealed intense competition for resources such as food availability that resulted in biphasic boom and bust nematode population dynamics (*44*). Therefore, Eu gonochoristic species may exploit a specific element of these ecological dynamics, despite the cost to mating chance.

Cannibalism in *Pristionchus* is part of a complex intraguild predation behavior, as these worms can kill and feed on various nematodes, likely as a means to both remove rivals competing for the same resources and acquire extra nutrients (*33*, *34*). In addition, they are capable of repelling adult potential competitors from their territory through the avoidance response generated from a predatory bite on an adult cuticle (*14*). While previous studies revealed cannibalism behaviors between geographically distant strains but not on self-progeny due to a self-recognition system (*28*), we have now been able to demonstrate that this behavior is ecologically relevant and depends on more than just the previously identified self-recognition component *self-1*. Furthermore, the self-identification mechanism extends to more than just self-progeny, as it also includes close kin. The complexity of the mechanism is also likely to expand beyond a single gene involving components across the whole genome. Thus, in the predatory genus *Pristionchus*, the evolution of plasticity, genomic relatedness, and self-recognition systems enable the existence of cannibalistic behaviors. Therefore, we propose developmental plasticity as a general principle that influences cooperative versus competitive social action strategies in different animal systems from nematodes to vertebrates.
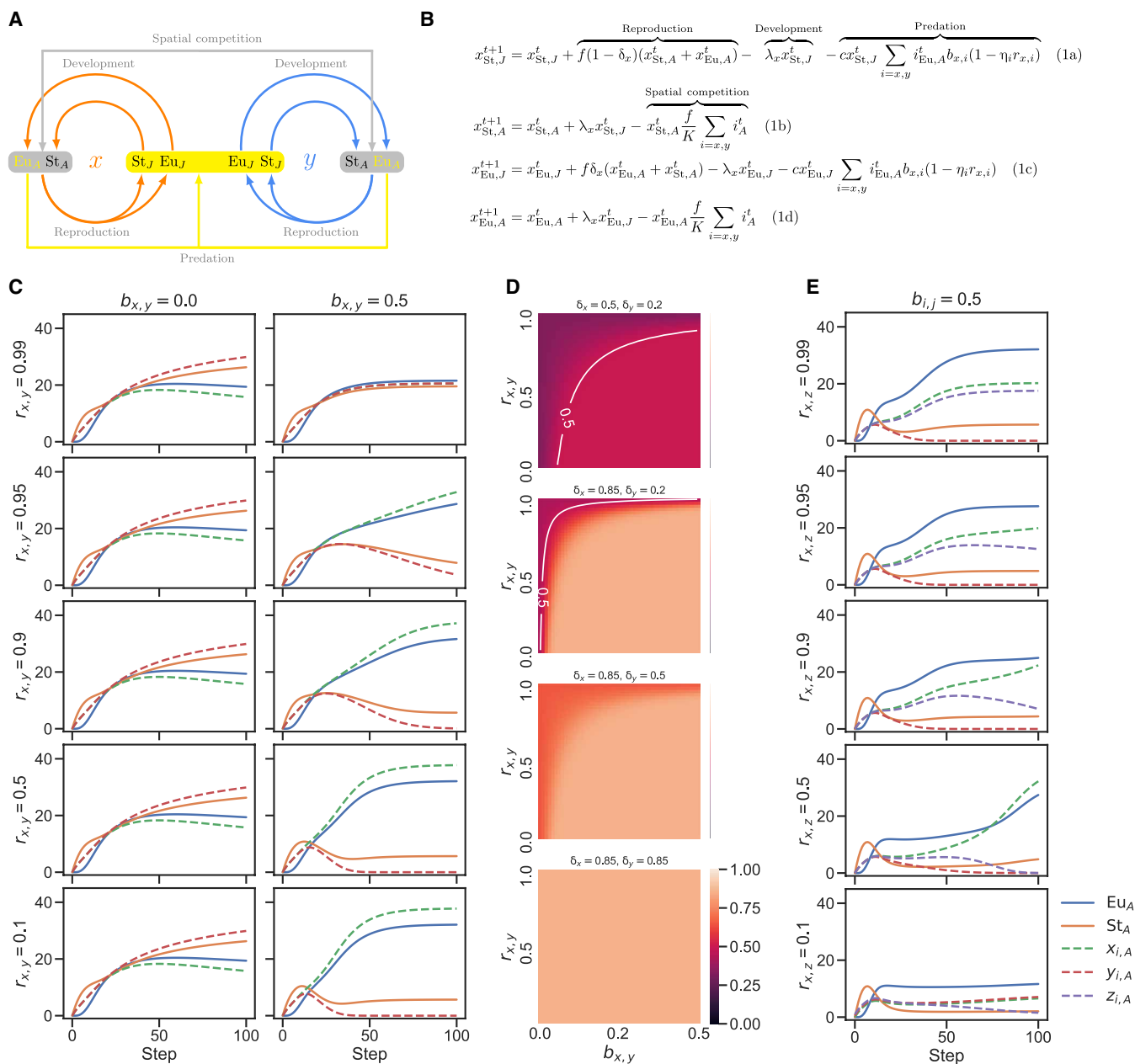
Fig. 3. Predation of hermaphrodites fits a selfing model of cannibalism. (**A**) Diagram of variables used throughout the model. (**B**) Dynamical equations solved for generating models. Genotype x dynamics follows four equations, 1a to 1d, with carrying capacity (K) and cost of predation (c). Genotype i adults will produce Eu juveniles with probability δ_i and St juveniles with probability $1 - \delta_i$. At each step, fraction λ develop into adults with adults producing f offspring. A fraction ($b_{j,i}$) of predatory encounters between juveniles of genotype j and Eu adults of genotype i is realized. The propensity of Eu adults to prey on juveniles decreases with increasing genomic relatedness (r), where $r_{i,j} = r_{j,i}$ and two individuals of the same genotype are fully related ($r_{i,i} = 1$). Predation depends on the genotype-dependent ability for kin recognition (η_i). Dynamics of genotypes y and z follow the same equations. (**C**) If genotypes do not interact ($b = 0.0$), the St-biased strain (y, $\delta_y = 0.2$) outnumbers the Eu-biased stain (x, $\delta_x = 0.85$). With high probability of encounter ($b = 0.5$), the St-biased genotype y can only coexist with genotype x if genetic relatedness is high and there is kin recognition. (**D**) Heatmaps representing the frequency of Eu adults after 100 steps under different combinations of r and b. The majority of conditions result in a strong Eu bias. The white contour line indicates when 50% of adults are of Eu after 100 steps. (**E**) Three-genotype selfing model, given $\delta_x = \delta_y = \delta_z = 0.85$. The coexistence of x, y, and z under high encounter ($b = 0.5$) is determined by pairwise relatedness ($r_{xy} = 0.2$, $r_{yz} = 0.1$). x and z outcompete y, reinforcing the role of relatedness. Results of (C), (D), and (E) are obtained by numerically solving Eqs. 1a to 1d for two and three-genotype cases. In all models, the initial number of juveniles of the St morph for each strain was 10, with other types set at 0 ($K = 50$, $\lambda = 0.1$, $\eta = 0.99$, $c = 0.1$, and $f = 0.6$).

MATERIALS AND METHODS
Nematode and bacterial strains
A list of all nematode species and strains can be found in table S1. Wild *P. pacificus* isolates from specific field sites on La Réunion were always frozen and stored within the first 10 generations after isolation to minimize domestication and thereby facilitate the investigation of interactions between ecologically relevant populations.

Nematode culture conditions
All nematode species and strains were grown under standard nematode growth conditions on nematode growth media (NGM) plates seeded with *Escherichia coli* OP50 and maintained at 20°C.

Mouth-form phenotyping
Mouth-form phenotyping was performed as previously reported (*27*). In brief, synchronized adults were placed onto a Discovery stereomicroscope with high magnification (×150). The Eu mouth form was determined by the presence of a wide mouth with two teeth, whereas the St forms were determined by a narrow mouth and a single tooth. Animals with the Eu mouth type were picked for predation assays.

Mouth form–reproductive mode statistical analysis
An association between mouth-form preference and reproductive mode was determined by fitting beta regression model using the R package betareg (*45*). Mouth-form ratios of some species represent 0 and 1 values. Thereby, we applied a $(y*(n − 1) + 0.5)/n$ transformation, where y is the response variable and n is the sample size (*46*). ANOVA test was run on the model using R package car (*47*). Phylogenetic analysis was performed on a modified Newick version of the *Pristionchus* phylogeny in Rödelsperger *et al.* (*29*). Phylogenetic signal was analyzed using R package phylosignal (*48*), based on the method of autocorrelation while using mouth-form ratios as a continuous trait.

Predation assays: Corpse assays
Corpse assays facilitated rapid quantification of predatory behavior and were conducted as previously described (*15*, *25*). Briefly, to generate substantial quantities of larvae for use as prey, *Pristionchus* strains were maintained on *E. coli* OP50 bacteria until freshly starved, resulting in an abundance of young larvae. These plates were washed with M9 buffer, passed through two Millipore 20-μm filters and centrifuged at 377*g* to form a concentrated larval pellet of juvenile animals. Excess buffer was removed, and 1 μl of worm pellet was deposited onto a 6-cm NGM-unseeded assay plates. This resulted in roughly 3000 prey larvae on each assay plate. Assay plates were left for a minimum of 1 hour to allow larvae to distribute evenly over the plate. Young adult *Pristionchus* predators were screened for the required mouth form and transferred to empty NGM plates for 30 min to remove any excess bacteria from their bodies. Subsequently, 20 *Pristionchus* predators were added to assay plates and allowed to feed for 24 hours before removal, and the plates were scored for the presence of corpses.

Population genomic analysis and relatedness
Raw RNA-seq reads were aligned to the *P. pacificus* reference genome (version El Paco) by the TopHat2 software (version 2.0.14, default settings) (*49*). Variable positions that were previously identified on the basis of population-scale whole-genome sequencing (*20*, *36*)

were called in the RNA-seq alignments with the samtools mpileup (version 0.1.18, default options) and bcftools view (version 0.1.17-dev, -cg options) programs (*50*). Between 10,000 and 70,000 single-nucleotide variant (SNP) positions with homozygous calls in either all samples or samples of a given population (variant quality score ≥ 20) were concatenated into pseudoalignments. These alignments were taken to calculate a neighbor-joining tree (*51*), representing the genome-wide phylogenetic relationships, and to calculate a percentage identity matrix that was further used to compute genome-wide relatedness. Percentage identity values were normalized by subtracting the population mean identity value. Genome-wide relatedness r was then obtained by dividing the normalized identity with the maximum absolute normalized identity value so that genetically identical pairs of strains have an $r = 1$, whereas the average $r = 0$ and pairs that are more distant than the average have an $r < 0$.

RNA-seq for identification of *self-1*
RNA-seq transcriptome data for all 36 *P. pacificus* strains were generated by first washing worms from three well-grown plates for each strain. These were pelleted down before resuspending in 1 ml of TRIzol. RNA was phenol-chloroform–extracted and cleaned using the RNA Clean & Concentrator Kits (Zymo Research) according to the manufacturer's guidelines. RNA was subsequently prepared using TruSeq RNA Sample Preparation Kit v2 (Illumina Inc.) according to the manufacturer's guidelines from 1 μg of total RNA in each sample. Libraries were quantified using a combination of Qubit and BioAnalyzer (Agilent Technologies) and normalized to 2.5 nM. Samples were subsequently sequenced as 150–base pair paired-end reads on multiplexed lanes of an Illumina HiSeq3000 (Illumina Inc.). RNA-seq data have been deposited at the European Nucleotide Archive under the study accession PRJEB41213. The *self-1* locus could be assembled in 33 of 36 strains by the Trinity software (version 2.2.0) (*52*). Several strains have multiple copies of the *self-1* locus as previously described (*28*). On the basis of the classification of assembled sequences into genes and isoforms by the Trinity assembler, copy number was determined as the number of Trinity genes with *self-1* homologs (as identified by TBLASTN searches).

Map generation
The La Reunion island map was generated using the software GeoMapApp (*53*).

Modeling
In our discrete-time model, the population consists of two stages, juveniles (J) and adults (A); two morphs, predatory (Eu) and nonpredatory (St); and $n \geq 2$ genotypes. At each time step, a fraction λ of all juveniles in the population develop into adults, and the remaining fraction $1 − λ$ persist in the juvenile stage, while every adult produces f new juveniles. Morphs are genetically determined at birth: For an adult of genotype i, a fraction $δ_i$ of the produced juveniles f is of the Eu morph, while the remaining fraction $1 − δ_i$ is of the St morph. Resource competition between adults due to limited environmental carrying capacity K leads to adult mortality in proportion to total adult densities. The only cause of death for juveniles is predation. Preys (juveniles) and predators (Eu adults) meet in proportion to their densities. For a juvenile of genotype j and a Eu adult of genotype i, the risk of predation upon an encounter is $b_{j,i}$. The propensity of an Eu adult to actually prey decreases with increasing genomic relatedness (r) with the encountered juvenile, where $r_{i,j} = r_{j,i}$

and two individuals from the same genotype are fully related, i.e., $r_{i,i} = 1$. The adult predation propensity also depends on the genotype-dependent ability in kin recognition (η_i). We assume equal recognition abilities for all genotypes ($\eta_i = \eta_j$ for any two genotypes i and j). The parameter c sets the genotype-independent overall frequency of predator-prey interactions, and it can be interpreted as the cost of predation.

In the simplest scenario, there are only two genotypes x and y. On the basis of the aforementioned life cycle, the dynamics of the subpopulation of genotype x is

$$
x_{\text{St},J}^{t+1} = x_{\text{St},J}^{t} + \overbrace{f(1-\delta_x)(x_{\text{St},A}^{t}+x_{\text{Eu},A}^{t})}^{\text{Reproduction}} - \overbrace{\lambda x_{\text{St},J}^{t}}^{\text{Development}} - \quad (1a)
$$
$$
\underbrace{c\, x_{\text{St},J}^{t} \sum_{i=x,y} i_{\text{Eu},A}^{t}\, b_{x,i}(1-\eta_i r_{x,i})}_{\text{Predation}}
$$

$$
x_{\text{St},A}^{t+1} = x_{\text{St},A}^{t} + \lambda x_{\text{St},J}^{t} - \overbrace{x_{\text{St},A}^{t}\frac{f}{K}\sum_{i=x,y} i_A^{t}}^{\text{Spatial competition}} \quad (1b)
$$

$$
x_{\text{Eu},J}^{t+1} = x_{\text{Eu},J}^{t} + f\delta_x(x_{\text{St},A}^{t}+x_{\text{Eu},A}^{t}) - \lambda x_{\text{Eu},J}^{t} - \\
c\, x_{\text{Eu},J}^{t} \sum_{i=x,y} i_{\text{Eu},A}^{t}\, b_{x,i}(1-\eta_i r_{x,i}) \quad (1c)
$$

$$
x_{\text{Eu},A}^{t+1} = x_{\text{Eu},A}^{t} + \lambda x_{\text{Eu},J}^{t} - x_{\text{Eu},A}^{t}\frac{f}{K}\sum_{i=x,y} i_A^{t} \quad (1d)
$$

The dynamics of the subpopulation of genotype y are analogous to Eqs. 1a to 1d. In the n genotype case, the subpopulation of genotype j is described by dynamical equations as in Eqs. 1a to 1d, with the only difference that both the predation term and the spatial competition term are generalized. A software used to numerically solve the recursive equations of our model was written in Python 2.7 and is available at https://github.com/Kalirad/asexual_plastic_model.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/7/35/eabg8042/DC1

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Sex or cannibalism: Polyphenism and kin recognition control social action
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