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Specialist versus generalist life histories and nucleotide diversity in *Caenorhabditis* nematodes

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Species with broad ecological amplitudes with respect to a key focal resource, niche generalists, should maintain larger and more connected populations than niche specialists, leading to the prediction that nucleotide diversity will be lower and more subdivided in specialists relative to their generalist relatives. This logic describes the specialist-generalist variation hypothesis (SGVH). Some outbreeding species of *Caenorhabditis* nematodes use a variety of invertebrate dispersal vectors and have high molecular diversity. By contrast, *Caenorhabditis japonica* lives in a strict association and synchronized life cycle with its dispersal host, the shield bug *Parastrachia japonensis*, itself a diet specialist. Here, we characterize sequence variation for 20 nuclear loci to investigate how *C. japonica*'s life history shapes nucleotide diversity. We find that *C. japonica* has more than threefold lower polymorphism than other outbreeding *Caenorhabditis* species, but that local populations are not genetically disconnected. Coupled with its restricted range, we propose that its specialist host association contributes to a smaller effective population size and lower genetic variation than host generalist *Caenorhabditis* species with outbreeding reproductive modes. A literature survey of diverse organisms provides broader support for the SGVH. These findings encourage further testing of ecological and evolutionary hypotheses with comparative population genetics in *Caenorhabditis* and other taxa.

1. Introduction

An essential aim in evolutionary genetics is to relate the patterns of genetic diversity with causative evolutionary forces and with features of organismal ecology and life history [1,2]. When it comes to life-history traits affecting resource use, species lie on a continuum between generalists (i.e. large array of resources) and specialists (i.e. narrow set of resources). However, defining specialist and generalist species is not straightforward, because the marginal importance of a particular resource across an organism's lifetime or niche space is not always obvious, owing to context-dependent or developmentally restricted strategies [3]. Moreover, if generalist populations consist primarily of specialized individuals [4], then generalist strategies may be transient with specialization leading to speciation [3]. Nevertheless, the evolution of specialist–generalist strategies seems to be highly dynamic and may play important roles in species diversification [5–7]. We use the terms specialist versus generalist to refer to life-history traits relating to some key resource that is expected to constrain long-term population size.

Because of their specialization in resource use, the habitat of specialist species may be more heterogeneous and patchier than for generalists. Populations of specialists may therefore be less connected and more subdivided into smaller populations than generalists. For instance, a meta-analysis found a significant positive correlation between niche breadth and geographical range, suggesting that populations of generalists may be larger than specialists [8]. Resource generalism also may promote the persistence of populations in the

long term [5], because generalists could be less sensitive to stochastic fluctuations of any given resource by being able to replace one scarce resource with another. Specialists, therefore, may be more subject to meta-population dynamics, with repeated bottlenecks further decreasing the effective population size of already smaller populations [9]. Moreover, such meta-population dynamics could cause a higher extinction risk for specialists [10]. Thus, specialists are expected to have lower effective population sizes than generalists, and to be composed of populations with less gene flow between them. We refer to this logic as the specialist–generalist variation hypothesis (SGVH). Consistent with these predictions, a survey of the literature indicates that generalist species tend to have more molecular diversity than related specialist species: in 11 of 14 studies we identified, the specialist species showed lower genetic diversity than related generalists (table 1). A classic meta-analysis of allozyme data similarly reported the same difference between habitat specialists versus generalists in plants, invertebrates and vertebrates [23]. However, subsequent investigations of allozyme variation found equivocal support for the SGVH [19–21,24]. A caveat to interpretation of allozyme datasets, however, is that they do not strictly summarize neutral population genetic variability to connect to effective population size, because selection may act on protein variants. The scarcity of sequence-based estimates of genetic diversity thus makes them especially valuable.

The SGVH idea is superficially related to the niche variation hypothesis (NVH), which proposes that adaptation to the exploitation of multiple resources, made possible by lower interspecific competition, will drive evolution of greater morphological diversity within species so that species with broader niches are predicted to have higher morphological variation [25]. While the NVH offers an adaptive explanation for differences in phenotypic variation, the SGVH aims to describe neutral genetic variation as a connection to population size and population differentiation. Analyses of diet use provide strong support for the NVH, manifesting through among-individual differences in diet rather than breadth of individual diets [4,26,27]. Nevertheless, niche expansion does not necessarily result in greater morphological variation, and phenotypic variation may not have a heritable basis [4,27].

Nematodes of the genus *Caenorhabditis* show striking life-history differences that are expected to influence effective population size [28]. For example, distinct reproductive modes among species (obligatorily outbreeding versus near-complete self-fertilization) provide one well-studied axis to which this powerful genetic model system has been applied in evolutionary biology [29–31]. Here, we focus on a less-emphasized life-history difference: disparities in dispersal vector specialization. A diversity of phoretic hosts facilitate long-range dispersal for many *Caenorhabditis* species as the worms seek more favourable conditions and their food source: bacteria within decomposing vegetal matter [28,32–34]. For instance, *Caenorhabditis remanei* associates with several isopod species, *C.* sp. 5 has been found on snails and isopods, *Caenorhabditis plicata* on several groups of carrion beetle, *Caenorhabditis elegans* from isopods, gastropods, insects and millipedes, and *Caenorhabditis briggsae* is found on gastropods and insects [35–38]. By contrast, *Caenorhabditis japonica*, a gonochoristic (i.e. dioecious) species endemic to Japan, forms a specialized phoretic association with its dispersal host, the shield bug *Parastrachia japonensis*, such that the nematode life cycle synchronizes with that of the insect [39]. The shield bug is also a specialist species, feeding only on the fruits of the tree *Schoepfia jasminodora*, and having synchronized its own life cycle such that its reproductive period matches fruit availability [40]. The natural history of *C. japonica* is probably the best understood for any species of *Caenorhabditis* (figure 1*a* and Material and methods), and thus provides a unique opportunity to investigate patterns of genetic variation in relation to its ecology and specialized association with its host.

In this study, we report, to our knowledge, the first population genetic analysis of *C. japonica* and examine how its distinctive life-history traits affect patterns of nucleotide diversity. In particular, we evaluate the predictions that the specialized phoretic association of *C. japonica* leads to lower nucleotide variation and stronger genetic differentiation among populations. We also contrast diversity in species with distinct reproductive modes (outbreeders versus self-fertilizers). Our analysis is consistent with the SGVH prediction for levels of nucleotide variation, but not for population differentiation, and also affirms that outbreeding species are more genetically diverse.

2. Material and methods

(a) Study system

Caenorhabditis japonica nematodes participate in a tight phoretic association with a single insect host, the shield bug *P. japonensis*. During most of the year, the insect males and females aggregate in a quiescent non-feeding state on the leaves of non-host trees [41]. In the spring (May), most *P. japonensis* individuals then disperse for mating. Males die soon after mating, but females persist and make nests in the leaf litter in which they perform maternal care until the end of July by provisioning their young with drupes of their sole host tree, *S. jasminodora* [40].

During most of the year, *C. japonica* are found in clumps of tens of dauer larvae around the scutellum of female *P. japonensis* as the insects aggregate in reproductive diapause on non-host trees (figure 1*a*) [39]. The dauer 'quiescent' larval stage is entered as a facultative developmental pathway instead of direct development, with individuals being long-lived, resistant to stress, non-feeding and capable of nictating dispersal behaviour [42]. Dauers are not found on *P. japonensis* males in the field [39], despite their ability to associate with males, suggesting that they do not survive long on males [43]. Dauers can survive for several months associated with their host in a partially desiccated state [44], but have low survivorship compared with the dauers of other *Caenorhabditis* species if removed from their host, potentially owing to high oxidative stress [45]. *Caenorhabditis japonica* dauers survive only approximately 10 days without their host, suggesting that signals from the insect are necessary to maintain the worm in a quiescent state and underlie the obligatory association of *C. japonica* with *P. japonensis* [45].

Female insects still carry the dauer worms during their reproductive period, from May to June [39]. But high humidity and the presence of *S. jasminodora* fruits in the female litter nest, favoured by the rainy season from June to July, enables dauer worms to recover and resume their development [39,44]. *Caenorhabditis japonica* propagates to large numbers in and around the nest when insect nymphs hatch out, with very few dauers remaining on parent females after eggs hatch [39,46]. Multiple generations of propagating stages of *C. japonica* are found feeding on bacteria in the rotten fruits of *S. jasminodora* as well as on dead nymphs and egg carcasses [39,46]. By contrast, dauers continuously embark on newly hatched nymphs and new adult insects [39], using chemical attractants secreted by the insect host for recognition [43] and engaging in nictation behaviour (i.e. lifting and

Table 1. Comparison of nucleotide diversity and population differentiation between generalist and specialist species. (CO, cytochrome oxydase; mtDNA, mitochondrial DNA; AFLP, amplified fragment length polymorphism; RAPD-PCR, random amplification polymorphic DNA-polymerase chain reaction; RFLP, restriction length polymorphism; n.a.: not available.)

organisms[a]	trait	data	lower nucleotide variation	higher population differentiation	reference
nematodes (*Caenorhabditis*: 3)	dispersal host specificity	20 nuclear sequence loci	specialist	generalist	this study
Platyhelminthes (*Lamellodiscus*: 7)	host specificity	ribosomal transcribed spacer 1	specialist	n.a.	[11]
fruit flies (*Drosophila*: 29)[c]	diet specificity	nucleotide sequence	specialist	n.a.	[1]
ground beetles (*Carabus*: 2)	habitat specificity	microsatellites	generalist	specialist	[12]
milkweed beetles (*Chrysochus*: 2)	diet specificity	RFLP and sequencing of mtDNA	specialist	generalist	[13]
bark beetles (*Dendroctonus*: 2)	diet specificity	three allozyme loci and mtDNA-RFLP	specialist	specialist	[14]
moths (*Heliothis*: 2)	diet specificity	AFLP	generalist	specialist	[15]
aphids (*Neuquenaphis*: 4)	diet specificity	RAPD-PCR markers	specialist	specialist	[16]
wild bees (*Caupolicana*: 1; *Leioproctus*, 1; *Nolanomelissa*: 1; *Trichothurgus*: 1; *Centris*: 2; *Cadeguala*: 1; *Colletes*: 1; *Acamptopoeum*: 1; *Neofidelia*: 1)	pollen diversity	26 or 14 allozyme loci	specialist	n.a. or specialist	[17,18]
marine gastropods (*Monodonta*: 2; *Gibbula*: 1)	habitat heterogeneity	26 allozyme loci	specialist	n.a.	[19]
marine gastropods (*Littorina*: 2)	habitat heterogeneity	17 allozyme loci	specialist	n.a.	[20]
marine teleosts (106 species)	habitat specificity	allozymes	generalist	n.a.	[21]
butterflyfish (*Chaetodon*: 2)	diet specificity	microsatellites and mtDNA	specialist	generalist	[22]

[a]Genus names and number of species studied are given in parentheses.
[b]Diet specialist *Drosophila sechellia* has the lowest nucleotide polymorphism of all *Drosophila* species reported in [1].

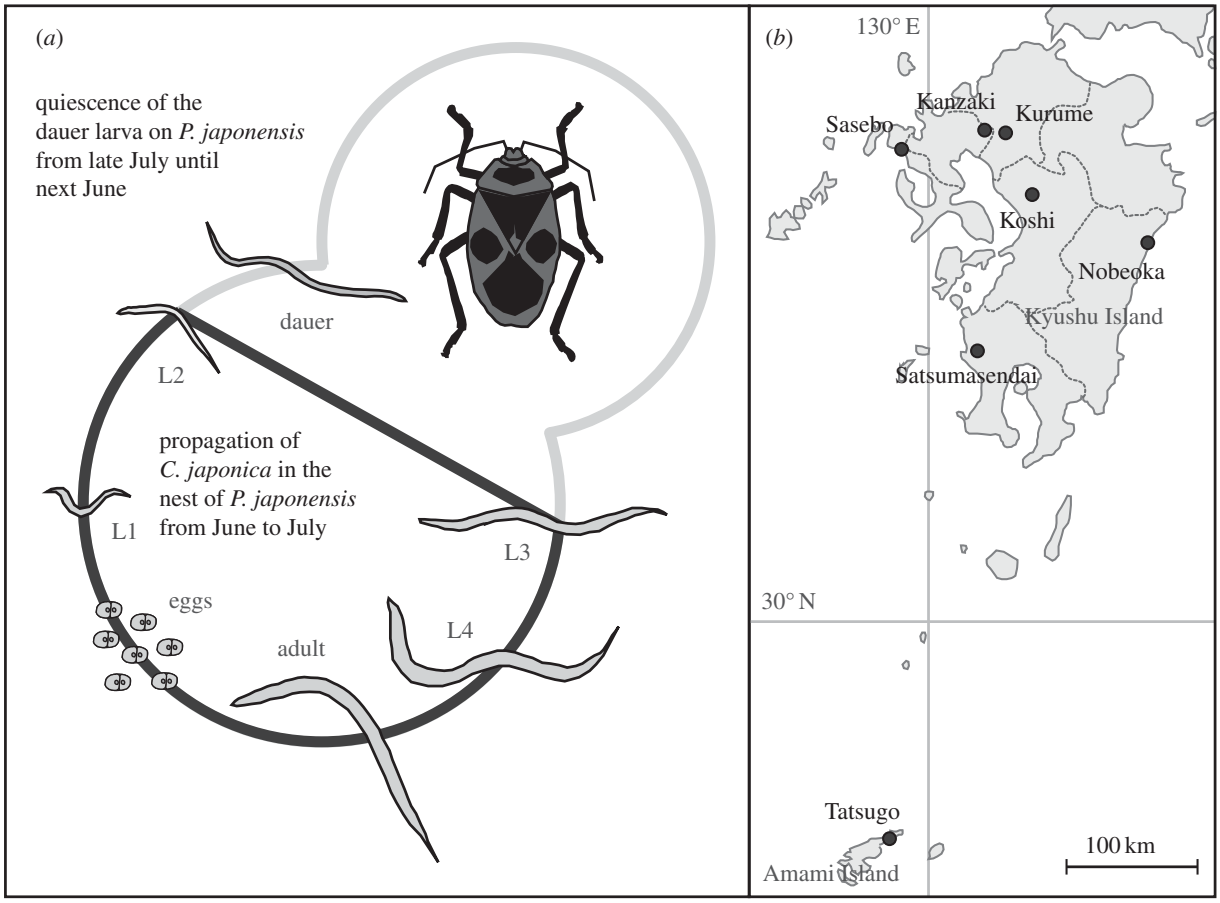

Figure 1. (*a*) *Caenorhabditis japonica* life cycle. *Caenorhabditis japonica* dauer larvae disembark from the females of *P. japonensis* and proliferate in the bug's nest during the rainy season (June to late July). In late July, dauer larvae embark on newly emerged *P. japonensis* adults and enter a quiescent stage, waiting for the next spring to resume their development. L1–L4: first–fourth larva stage. (*b*) Locations of sampled populations of *C. japonica*.

waiving of the body) and negative gravitactic behaviour (i.e. swimming upwards) for host finding [47]. Parental insect females die by the time nymphs reach the third of their five larval stages. Newly formed adult insects emerge in late July and, carrying *C. japonica* dauer larvae, join aggregations with old adult insects on non-host trees until they resume their life cycle in the spring of the next year (figure 1*a*).

(b) Sampling, strains and rearing conditions

Insect hosts were collected opportunistically from aggregations on non-host tree leaves at seven localities on two islands of southern Japan (figure 1*b*; electronic supplementary material, table S1). Sacrificed bugs were individually placed on a Petri dish with Nematode Growth Medium (NGM) seeded with *Escherichia coli*, in which *C. japonica* dauers were allowed to recover for a couple of days. Female nematodes with a copulatory plug, having mated with one or more males, were then individually picked and placed on a freshly seeded NGM plate in order to establish strains as isofemale lines. Nematodes were maintained at 25°C on NGM plates or lipid agar plates [48]. With a few exceptions, we used just a single strain isolated from each host to quantify nucleotide variation. Details for each of the 51 strains are listed in the electronic supplementary material, table S1, including sampling locality, host carrier, collection date and loci sequenced; although not all strains were successfully cryopreserved.

(c) DNA amplification and sequencing

Genomic DNA was amplified from a single male for each strain, or from a single female if no live males were available, by picking the animal directly into a Repli-G kit reaction (Qiagen). We then PCR amplified and sequenced 20 nuclear loci from the genomic template DNA, using primers designed from the *C. japonica* genome assembly for orthologues of X-linked protein-coding genes in *C. elegans* (see the electronic supplementary material, table S2). These putatively X-linked loci were selected to minimize intra-individual heterozygosity (males are hemizygous for the X chromosome). We used a second set of primers when amplifications failed and sequenced both amplicon strands at the University of Arizona UAGC sequencing facility. The number of strains successfully sequenced per locus ranges from 41 to 51 (see the electronic supplementary material, table S1). Amplifications were processed in 30 µl reaction volumes with 1.5 µl dimethyl sulfoxide, 3 µl dNTPs (6.6 mM), 3 µl 10X buffer (Fermentas), 2.4 µl $MgCl_2$, 0.36 µl of each primer (50 µM), 0.18 µl of Taq polymerase (New England Biolabs) and 2 µl of genomic DNA. Cycling conditions were: 95°C for 4 min followed by 35 cycles of 95°C for 1 min, 55°C, 58°C or 60°C for 1 min and 72°C for 1 min. All polymorphisms were visually verified using sequencing chromatograms with heterozygous sites coded using International Union of Pure and Applied Chemistry guidelines. Haplotypes from female samples or loci that appeared not to be X-linked were resolved using the program PHASE v. 2.1 implemented in DnaSP v. 5.10 [49]. Primer sequences were manually deleted from each sequence prior to analysis. All new sequences are available in Genbank under accession numbers KF579910–KF580843.

(d) Sequence analyses

We generated multiple sequence alignments manually for each locus using Bioedit [50] and then measured nucleotide polymorphism [51] for each locus using DnaSP v. 5.10 [49]. We then combined these data with published polymorphism data

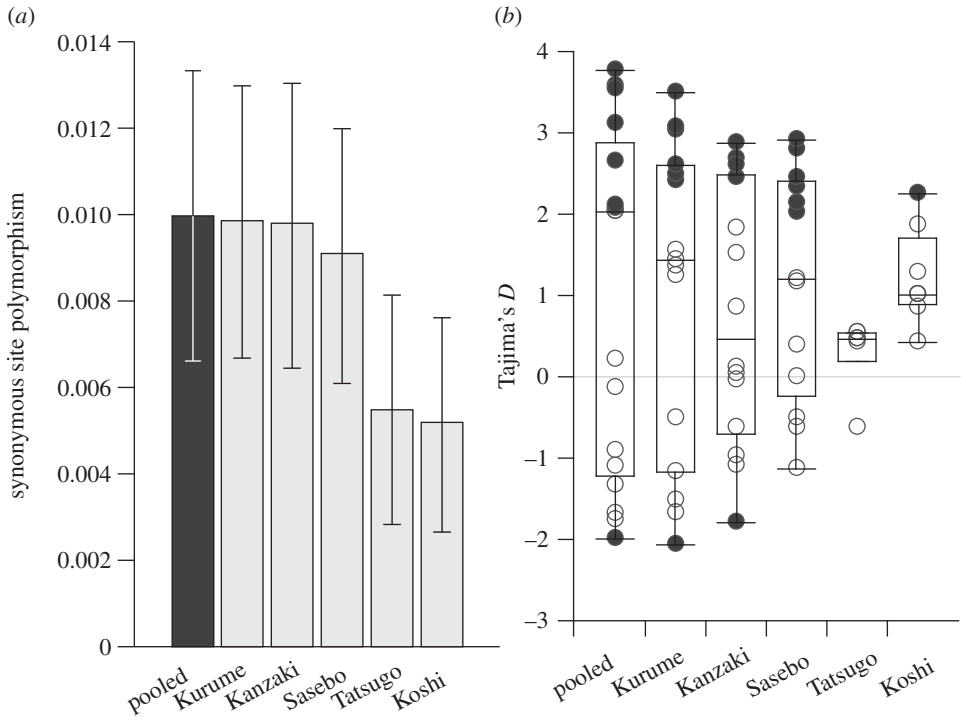

Figure 2. (*a*) Similar levels of nucleotide diversity in different populations of *C. japonica*. Means with same letters are not significantly different (Wilcoxon two-sample). Means are represented with ± 1 s.e. (*b*) Tajima's *D* measures of the site frequency spectrum tend to be positive in each population of *C. japonica*. Boxplots show the median *D*-value and interquartile range. Values of *D* showing significant deviation from neutrality are represented by dark grey circles.

for other *Caenorhabditis* species to contrast diversity between mating systems ([52] and references therein). We quantified the site frequency spectrum with Tajima's *D* (D_{Taj}) [53] in DnaSP v. 5.10 using either synonymous or silent sites. For genes CJA16551 and *Cja-lfi-1*, however, we computed D_{Taj} using polymorphism at non-synonymous sites because no silent site polymorphisms were present. We measured genetic differentiation and distance among populations using F_{ST} [54] and D_{xy}, the average number of nucleotide substitutions per site between groups [51]. We then examined patterns of isolation by distance by plotting F_{ST} as a function of the geographical distance between sampling sites, estimated linearly between two locations. Because of missing sequence data for some loci in some individuals, we discarded three loci (*Cja-apa-2*, *Cja-dpyd-1* and *Cja-mrp-4*) that were among the least variable ($1 \leq S \leq 3$) and concatenated the 14 remaining loci to infer the relationship among 33 of the 51 strains with an unrooted neighbour-network generated with a Jukes–Cantor distance in the program SplitsTree v. 4.10 [55].

3. Results

(a) Nucleotide diversity

If life-history traits influence long-term population size, then we expect that this will be reflected in population genetic patterns of neutral polymorphism because the neutral theory of molecular evolution predicts a direct relationship between effective population size and neutral genetic variation [56,57]. The 12.3 Kb of sequence per strain we analysed for *C. japonica* contained a total of 19 replacement-site single nucleotide polymorphisms (SNPs) and 113 silent-site SNPs. Pooling the strain samples from seven locations on two different islands of Japan (figure 1*b*), we found an average SNP density at silent sites of $\pi_{\mathrm{si}} = 0.97\%$ and at replacement sites of $\pi_{\mathrm{aa}} = 0.036\%$ for these 20 nuclear loci (see the electronic supplementary material, table S3). However, the global pattern of nucleotide variation is not greatly influenced by this pooled sampling scheme. Within each of the three local population samples containing 10 or more strains, nucleotide polymorphism is equivalent to the pooled species average (figure 2*a*); two local populations with low sample size have nominally, but not significantly, lower diversity (figure 2*a*). This nucleotide diversity in *C. japonica* is 3.7-fold–16.5-fold less than that observed previously for outcrossing *Caenorhabditis* species (ANOVA $F_{4,107} = 23.5$, $p < 0.0001$; Tukey's post-hoc comparisons), which show molecular hyperdiversity (figure 3) [58]. Additionally, *C. japonica* diversity is a nominally 2.5-fold higher than that seen for selfing species [52,58] (figure 3).

(b) Population differentiation

A secondary prediction of the SGVH holds that specialist life histories will be associated with greater habitat fragmentation leading to less gene flow among populations. We investigated this possibility in *C. japonica* by quantifying the pattern of genetic distance among individuals and among populations. First, we inferred the relationships of genetic distance among *C. japonica* strains and examined whether they group according to sampling localities. The neighbour-joining network shows that strains partition loosely into four clusters, but that no clear association emerges between genetic clustering and geographical origin (figure 4). Second, we measured the genetic distance between populations using D_{xy} and found that, on average, each pair of populations has similar pairwise nucleotide divergence (table 2). Third, we quantified genetic differentiation between populations using the F_{ST} statistic. Overall, *C. japonica* populations with sample sizes of at least 10 are not strongly isolated in terms of gene flow (table 2). When we plotted F_{ST} as a function of geographical distance to test for isolation by distance, we observed no significant correlation (Mantel test, $p = 0.53$; electronic supplementary

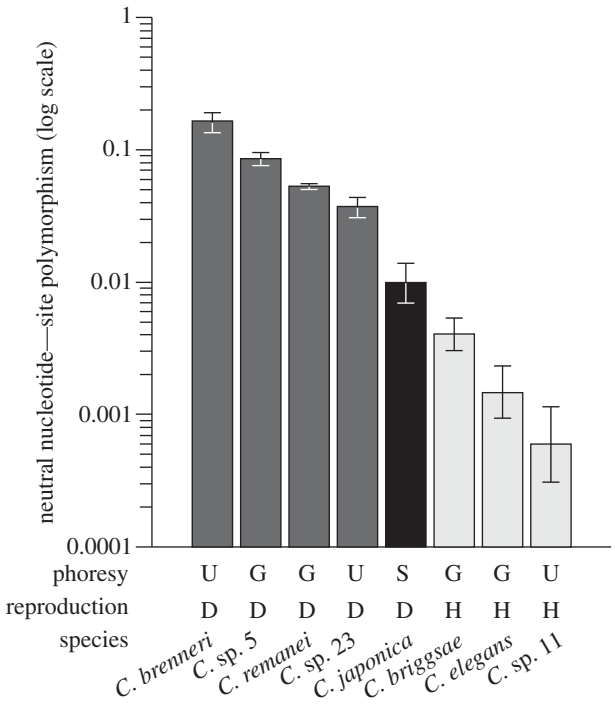

Figure 3. Nucleotide diversity in *Caenorhabditis* is strongly influenced by the mating system. Outcrossing species (dark grey and black) have on average more nucleotide polymorphism at neutral sites than hermaphroditic species (light grey). Means are represented with ± 1 s.e. The mode of reproduction and the type of phoretic association are indicated for each species. D, dioecious; H, hermaphroditic; G, generalist; S, specialist; U, unknown.

material, figure S1). We conclude that populations of *C. japonica*, at least in the area sampled, are not strongly disconnected by gene flow.

(c) Deviation from neutral patterns of sequence variation

Population demography that differs from the standard neutral model leaves a signature in the genome by perturbing the site frequency spectrum of variants from neutral expectations. We used Tajima's *D* statistic (D_{Taj}) to quantify the site frequency spectrum of each locus both across sampling sites and within each population. At the species level, the distribution of D_{Taj} for pooled samples is skewed towards positive values (median $D_{\mathrm{Taj}} = 1.49$), with D_{Taj} significantly greater than 0 for 7 loci (figure 2*b* and electronic supplementary material, table S3), indicating an overabundance of variants with intermediate frequency relative to the expected distribution of variant frequencies under the standard neutral model [53]. Just one locus shows a significant excess of rare alleles (*Cja-cht-1*; electronic supplementary material, table S3). Moreover, the distribution of D_{Taj} across loci is bimodal with wide variance, further indicative of historical population demography that deviates from the standard neutral model. Finally, the pattern of variant frequencies within each local population of *C. japonica* is consistent with the pattern of a deficit of rare variants observed for the pooled sample of strains (figure 2*b* and electronic supplementary material, tables S4–S8). Thus, analyses of the site frequency spectrum indicate a poor correspondence of the standard neutral model to recent *C. japonica* demographic history. Several processes could yield both high variance and positive skews in the site frequency spectrum, including genetic admixture among subpopulations and/or a declining population size [59,60].

(d) Comparative population genetics of orthologues in *Caenorhabditis japonica* and *Caenorhabditis remanei*

Here, we compare directly the patterns of genetic variation for 11 orthologous loci common to our results for the phoretic host specialist *C. japonica* and to published data for the phoretic generalist *C. remanei* [61]. The restriction to orthologous loci ensures that the selective constraints on loci are as similar as possible between the two species. *Caenorhabditis remanei* is a cosmopolitan species found in temperate latitudes in North America, Europe and Asia, including Japan, and displays a generalist phoretic association with snails and as many as five isopod species [38,62]. However, its generalist phoretic association is not simply opportunistic, because *C. remanei* discriminates among host carriers and is never found associated with the isopod *Porcellio spinicornis*, a close relative to one of its hosts *Porcellio scaber* [38].

Consistent with results obtained with the larger dataset (figure 3), nucleotide variation at synonymous sites is 3.2-fold lower in *C. japonica* than in *C. remanei* for strictly orthologous loci and similar sample size (see the electronic supplementary material, figure S2 and table S9). Similarly, synonymous site polymorphism is more than twofold lower within populations of *C. japonica* than in populations of *C. remanei* (see the electronic supplementary material, figure S2 and table S9). Moreover, the site frequency spectrum differs substantially between the two species, suggesting different demographic dynamics between them. D_{Taj} values are much more positively skewed in *C. japonica* (median $D_{\mathrm{Taj}} = 2.381$) than in *C. remanei* (median $D_{\mathrm{Taj}} = 0.608$) for this set of orthologous genes (see the electronic supplementary material, tables S10 and S11). Specifically, the deficit of rare alleles implied by $D_{\mathrm{Taj}} > 0$ is significant for six out of the 11 loci in *C. japonica* but for none in *C. remanei*. These pooled patterns of allelic variation are similar to those observed within each population (excepting the German population of *C. remanei* [61]). The disparity between *C. japonica* and *C. remanei* in the amount of genetic variation at synonymous sites and in their frequency spectra suggests that their demographic histories differ substantially to influence effective population size.

Finally, we note that both species give significant negative D_{Taj} for the gene *cht-1* (*C. japonica*: $D_{\mathrm{Taj}} = -2.028$, $p < 0.05$; *C. remanei*: $D_{\mathrm{Taj}} = -2.096$, $p < 0.05$), compatible with the removal of neutral variation by hitchhiking effects of positive selection or by background selection effects of purifying selection. A role of selection in shaping variation at *cht-1* is plausible in light of its molecular function: it encodes a chitinase enzyme with a role in degrading cuticle during molting and could potentially be selected for a function in defence against chitin-containing pathogens.

4. Discussion

(a) The specialist–generalist variation hypothesis in relation to *Caenorhabditis japonica*

We demonstrate that populations of the phoretic host specialist nematode *C. japonica* contain many-fold lower genetic variability than all other known species in the genus that share an obligately outbreeding reproductive mode. Two of the

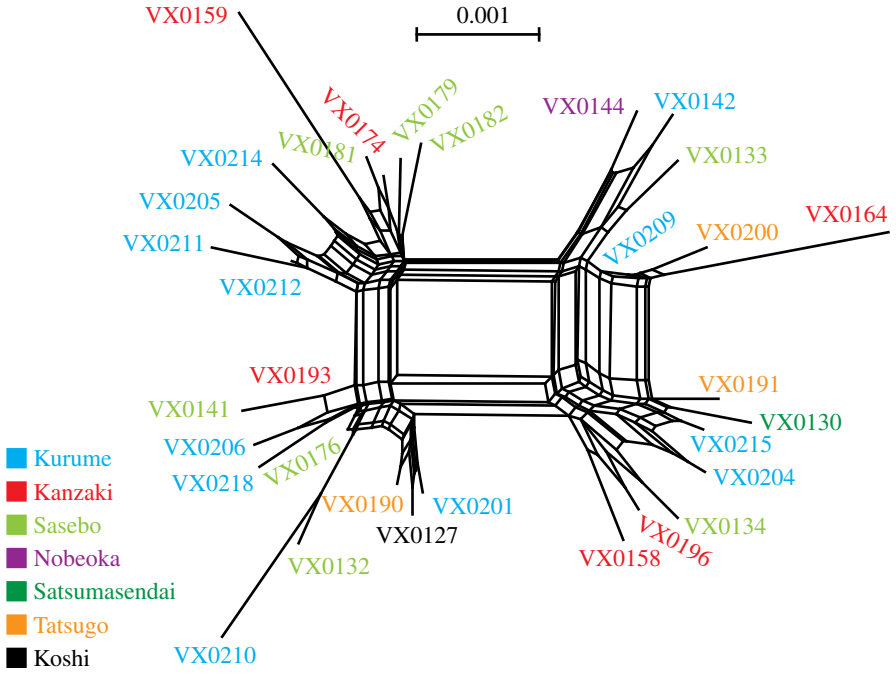

Figure 4. Neighbour-joining network based on concatenated sequences from 14 polymorphic loci showing the relationships among 33 *C. japonica* strains. Strains do not cluster according to their population of origin. Reticulation indicates recombination among strains. (Online version in colour.)

Table 2. Differentiation (mean F_{ST}, below diagonal) and per-site divergence (mean D_{xy}, above diagonal) between localities.

	Kanzaki[a]	Kurume[a]	Sasebo[a]	Tatsugo[b]	Koshi[b]
Kanzaki[a]		0.0037	0.0035	0.0035	0.0033
Kurume[a]	0.0329		0.0032	0.0036	0.0031
Sasebo[a]	0.0196	0.0249		0.0035	0.0026
Tatsugo[b]	0.1597	0.2416	0.2104		0.0037
Koshi[b]	0.0420	0.0954	0.0384	0.2562	

[a]Sample size $\geq$ 10.
[b]Sample size $<$ 5.

comparator species are known phoretic generalists (*C. remanei*, *C.* sp. 5); there is insufficient life-history data available to determine phoretic host fidelity for the two others (*C. brenneri*, *C.* sp. 23), although *C. brenneri* has been speculated to have unspecialized phoresy [63] and, like *C. remanei*, *C.* sp. 23 was found associated with isopods [61]. This pattern is consistent with the SGVH that species with specialist life histories, with respect to a trait that constrains population sizes, will have lower neutral genetic diversity than their generalist relatives. Although a stronger test awaits comprehensive phylogenetic comparative analysis, this finding joins a wide range of organisms from gastropods to insects to vertebrates that are compatible with the SGVH (table 1).

Caenorhabditis japonica's population genetic patterns implicate a complex demographic history, which has led to low polymorphism and SNP variants skewed towards intermediate frequencies both for pooled samples across its range and within local samples. Scenarios involving population contraction and genetic admixture among subdivided populations [53,59], as well as the consequences of biparental inbreeding for multiple generations within host nests, could plausibly contribute to these findings. Given these complex factors, we expect that genomic scale population data will be required to decipher the most likely demographic history. With marked contrast to *C. japonica*, populations of *C. remanei* appear closer to demographic equilibrium [61,64]. Specialist species may be more sensitive to variation in diet resources and changes in habitat conditions may impose population size bottlenecks more frequently than for generalists. The sole food resource for *C. japonica*'s host is typically scarce and is a major constraint for the survival of the developing *P. japonensis* nymphs [40]. Large variation in the quality and abundance of *S. jasminodora* drupes [40] suggests that seasonal and spatial variation in host insect abundance could correspondingly result in repeated bottlenecks of the populations of *C. japonica*. Meta-population dynamics in which local extinction and/or population size bottlenecks are followed by recolonization from nearby populations may be common in some *Caenorhabditis* [65] and would be accompanied by loss of species-wide nucleotide variation [66].

Because of the constraints imposed by their resource specialization, specialist species might live in fragmented habitats with populations separated by little gene flow. However, this secondary prediction of the SGVH receives limited support from our literature survey and we also found little evidence in *C. japonica* for strong population differentiation or of genetic isolation by geographical distance. This contrasts with the weak, but significant, differentiation and isolation by distance observed for the hyperdiverse and phoretic generalist species *C.* sp. 5 found in China [35,67].

A first caveat is that most *C. japonica* sampling sites are separated by relatively short distances (tens of kilometres; figure 1*b*); a second caveat is that the most distant population on Amami Island has a low sample size. This motivates additional sampling of *C. japonica*. Because *P. japonensis* congregate on non-host trees in a quiescent state during most of the year, their subsequent dispersal to establish individual nests could erase population subdivision across the range of *C. japonica*, a situation similar to the diet specialist butterflyfish [22]. Thus, the dispersal behaviour of hosts may not lead to strong gene flow barriers as presumed by a simple application of the SGVH for less vagile limiting resources.

We must also consider several non-mutually exclusive alternatives to the SGVH that could contribute to the observed patterns of nucleotide polymorphism in *C. japonica*. First, mutation rates are not known for the *C. japonica* genome, though the single nucleotide mutation rate does not differ between *C. elegans* and *C. briggsae* [68]. If the mutation rate per generation were several-fold lower in *C. japonica* compared with other species, then this could help account for lower absolute diversity because, at equilibrium, genetic variation is proportional to the rate of mutational input [57]. Second, we sampled *C. japonica* from its only described geographical home, Japan. However, the range of its host insect *P. japonensis* extends to the Yunnan province in China [69], and it is unknown whether the *C. japonica* range might extend to the Asian mainland. Thus, a founder effect with loss of heterozygosity in Japan could conceivably contribute to its seemingly low nucleotide variation compared with other *Caenorhabditis*. Third, coupled with its specialist habit, biparental inbreeding among only the tens of founding *C. japonica* individuals within host nests could depress total population diversity.

(b) Reproductive life-history and genetic variation in *Caenorhabditis*

Theory predicts profound negative effects on nucleotide polymorphism for selfing species relative to outcrossing ones [70–72], and we provide further support for this effect in *Caenorhabditis* [29,52,73,74]. Despite the lowest diversity reported to date among outcrossing species, *C. japonica* diversity is still nominally more than 2.5-fold higher, albeit not statistically significantly higher, than any of the three selfing species that evolved selfing hermaphroditism independently [28] (figure 3). Moreover, two selfing species are phoretic host generalists with worldwide geographical ranges (*C. elegans*, *C. briggsae*), suggesting that a selfing reproductive mode probably overpowers host specialization in constraining genetic diversity within species of *Caenorhabditis*.

5. Concluding remarks

The natural histories of most *Caenorhabditis* species are, as yet, insufficiently understood to determine their degree of dispersal vector specialization, though several species have specialized life histories. *Caenorhabditis angaria* has been found only in association with two weevil species, *C. bovis* is only isolated from the inflamed ear of zebu cattle, *C. plicata* associates only with carrion beetles of several species, and *C. drosophilae* has an obligatory phoretic association with *Drosophila nigrospiracula* [37]. These taxa provide intriguing avenues to further test for effects of specialization on nucleotide variation, from the perspective of both nematodes and their hosts, like *P. japonensis*. As high-throughput methods in molecular ecology become mainstream, we anticipate rekindled tests of old and new hypotheses for the connections between ecology, life history and population genetics in *Caenorhabditis* and other organisms [1].

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Data accessibility. Sequences are available in GenBank under accession nos. KF579910–KF580843.

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