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Host-finding behaviour in the nematode *Pristionchus pacificus*

Federico D. Brown*,†,‡, Isabella D'Anna† and Ralf J. Sommer

Department of Evolutionary Biology, Max Planck Institute for Developmental Biology, Spemannstrasse 37, Tübingen 72076, Germany

Costs and benefits of foraging have been studied in predatory animals. In nematodes, ambushing or cruising behaviours represent adaptations that optimize foraging strategies for survival and host finding. A behaviour associated with host finding of ambushing nematode dauer juveniles is a sit-and-wait behaviour, otherwise known as nictation. Here, we test the function of nictation by relating occurrence of nictation in *Pristionchus pacificus* dauer juveniles to the ability to attach to laboratory host *Galleria mellonella*. We used populations of recently isolated and mutagenized laboratory strains. We found that nictation can be disrupted using a classical forward genetic approach and characterized two novel nictation-defective mutant strains. We identified two recently isolated strains from la Réunion island, one with a higher proportion of nictating individuals than the laboratory strain *P. pacificus* PS312. We found a positive correlation between nictation frequencies and host attachment in these strains. Taken together, our combination of genetic analyses with natural variation studies presents a new approach to the investigation of behavioural and ecological functionality. We show that nictation behaviour in *P. pacificus* nematodes serves as a host-finding behaviour. Our results suggest that nictation plays a role in the evolution of new life-history strategies, such as the evolution of parasitism.

Keywords: ambush; behavioural genetics; body-waving; host attachment; nictation; *Pristionchus pacificus*

1. INTRODUCTION

Behavioural adaptations have been important in enabling nematodes to successfully exploit diverse habitats [1,2]. But little is known of the extent to which changes in behaviour influence the evolution of new life-history strategies in organisms.

One model used in foraging theory separates predatory and parasitic animals into two categories: cruisers and ambushers [3–5]. Cruisers are in constant movement and actively search for food (high energy cost), whereas ambushers tend to stand still and wait for their prey/host to approach (low energy cost) [3–5]. Infective juveniles of some ambusher nematode species show a specific search behaviour in which the animals stand on their tail and wave. This standing behaviour has been termed 'winken' [6], 'nictation' [7–9], 'standing' [10–12] and most recently 'body waving' [13]. For a complete revision about the terminology of this behaviour, see Kruitbos & Wilson [14]; for the sake of simplicity, we refer to this behaviour as nictation.

Nictation consists of raising the anterior and middle body regions of the juvenile off the ground, supported only by the tip of its tail [8,15]. In different species of nictating nematodes, juveniles can either stay in an erect pose or wave their bodies in three-dimensional spirals and loops [7,8,15]. In the laboratory, nictation behaviour is only observed when the nematodes are exposed to irregular substrates [13]. Foraging strategies of host finding in nematodes from the order Rhabditida have been extensively studied, e.g. in the parasitic *Steinernema* spp., *Heterorhabditis* spp. and *Phasmarhabditis hermaphrodita* because of their importance to pest management. The slug-parasitic nematode *P. hermaphrodita* attaches to hosts by crawling and is devoid of nictation, whereas insect parasitic *Steinernema* spp. attach by nictating [13,16]. Nictation behaviour has been also described in the animal-parasitic nematodes *Heligmosomoides polygyros* [17] and *Strongyloides ratti* [18]. By contrast, foraging strategies in the Diplogastridae, which are often associated with insects but do not necessarily parasitize them, have been poorly investigated. In this context, multiple questions can be addressed: do non-parasitic insect-associated nematodes show cruiser and/or ambusher behaviour? Is there a nictation-like behaviour? Such questions could best be addressed using laboratory model species in which behavioural assays could be complemented with genetic analyses.

Nematodes of the genus *Pristionchus* have a necromenic association with scarab beetles, in which arrested dauer-stage nematodes invade the insect, wait for the host to die and then resume development by feeding on growing micro-organisms on the carcass [19,20]. *Pristionchus pacificus*, a satellite model nematode for evolutionary and developmental studies, is known to live in association with scarab beetles in nature [21,22].

The *P. pacificus* community has up-to-date genetic and genomic tools available as well as transgenic techniques and the nematode is also amenable to studies of behaviour and neurobiology owing to the relative simplicity and detailed description of its nervous system (D. Bumbarger & R. J. Sommer 2011, personal communication). Therefore, the

*Author for correspondence (fd.brown46@uniandes.edu.co).
†Authors contributed equally.
‡Present address: Departamento de Ciencias Biológicas, Universidad de los Andes, Apartado Aéreo 4976, Bogotá, Colombia.

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features of *P. pacificus* allow us to address the question of whether nictation behaviour provides a selective advantage for nematode–host associations.

Molecular and morphological similarities support the hypothesis that the infective juvenile stage of parasitic nematodes is homologous to the dauer stage of non-parasitic nematodes, such as *Pristionchus* [23–26]. Harsh environmental conditions, such as high temperature, low food availability and high population density, induce many non-parasitic nematodes to develop into an alternative developmental juvenile stage referred to as 'dauer'. The dauer stage of *Pristionchus* species is responsible for host finding and attachment to host [20], and it is the only stage of development in which nictation occurs.

Nictation is proposed to provide a selective advantage that allows dauer juveniles to attach to passing hosts. On this basis, nictation could serve as an adaptation for the dauer juveniles to make contact with a host, in most cases an insect, for transportation, i.e. a phoretic interaction [27]. A consequence of a phoretic interaction is the permanent adherence of the dauer to the cuticle of its host, and in some cases cuticle penetration into body cavities of the host [28]. Therefore, phoresis has been suggested to serve as a pre-adaptation for the evolution of parasitism [9,23,24,26].

Herein by the use of an experimental set-up with *P. pacificus* strains, we tested the hypothesis that nictation behaviour favours the ability to attach to its beetle host with recent isolated and laboratory-generated mutant strains. Using forward genetics, we screened for mutants lacking nictation behaviour in *P. pacificus*. We isolated two mutants that can develop into proper dauers but are nictation-defective. We compared these two mutant strains with recently isolated strains from la Réunion island, with variable nictation rates, to test their ability to attach to artificial hosts under laboratory conditions.

2. MATERIAL AND METHODS

(a) *Nematode culture*

Breeding and maintenance of *P. pacificus* follow *Caenorhabditis elegans* standard culture methods that have been described previously [29,30]. Nematode strains used in this study were: (i) *P. pacificus* reference PS312 strain; (ii) *P. pacificus* RS5401 and RS5386, which are recent isolates from isogenic lines of *Oryctes borbonicus* scarab beetles or from soil samples from la Réunion island as described by Herrmann *et al.* [31]; and (iii) mutagenized *P. pacificus tu426* and *tu427*, which are nictation-defective strains isolated using a classic forward genetics approach [32]. In this study, we adopt the definition of strain described in Herrmann *et al.* [19]. Strain stocks were maintained on NGM plates with *Escherichia coli* OP50 lawns [29,30]. All *P. pacificus* strains used in this study are available upon request.

To generate dauers, we performed dauer inductions using the 'wet-plate method'. We resuspended three 6 cm NGM plates with fully grown mixed-stage worms into 1 ml of OP50 liquid medium and added it onto 10 cm NGM plates. Worms were grown at 20–25°C for approximately 14 days or until a sufficient number of dauers were found on the plate (A. Weller, 2009, personal communication). Timing of worms to dauer formation was investigated in *P. pacificus* PS312 and the two nictation-defective mutants *tu426* and *tu427*. We scored total hours necessary to reach the highest amount of dauers in the dauer induction plates.

Steinernema feltiae, *Steinernema carpocapsae* and *P. hermaphrodita* were supplied by Becker Underwood, UK. Dauer juveniles of *S. feltiae* and *S. carpocapsae* were cultured following Kaya & Stock [33]. Briefly, approximately 1000 *S. feltiae* or *S. carpocapsae* dauer juveniles were added to moistened Whatman filter paper in a 10 cm Petri dish and 5 to 10 *Galleria mellonella* were added. Plates were sealed with Parafilm and incubated at room temperature for 4 days. Once dead, then *G. mellonella* were transferred to White traps and dauers collected in the surrounding water. Nematodes were washed three times in M9 buffer before use and were stored at 4°C in 200 ml tissue culture flasks. *Phasmarhabditis hermaphrodita* (Nemaslug) were mixed with tap water (1 g in 100 ml) and stored similarly to *Steinernema* spp.

(b) *Nictation and attachment tests*

Nictation test arenas consisted of 6 cm NGM plates sparsely covered with sterile sand grains evenly spread with a shaker. Either 1000 or 5000 dauers, previously washed and resuspended in distilled water, were applied to the centre of the Petri dish and left to dry. The dishes were left covered (to preserve environmental conditions within the plate) at room temperature and nictation was recorded after the first hour and every 12 h thereafter for a period of 5 days. We used the first time point (1 h) to test for nictation differences between *P. pacificus* strains. To obtain the proportion of nictating dauers and dauers attached to host, we scored nictation activity for individual dauers that lifted their body off the substrate. For the host attachment studies, a single host was applied onto the plate already containing dauers and sand, prepared as described above. In two independent assays, we used 1000 and 5000 dauers, respectively, per plate and placed one *G. mellonella* moth larva (commercially available from HW Terra, Germany) on each plate; i.e. each replicate consisted of either 1000 or 5000 dauers/host/plate for each assay. All replicates contained the same stage, and the same size hosts. Nematodes were exposed to the host for a 2 hour period in covered plates. The hosts were removed, dissected and resuspended in water to allow release of dauers from the carcass. We counted the number of resuspended dauers.

(c) *Mutant screen*

We screened for nictation-defective mutant strains using EMS mutagenesis [32] in *P. pacificus* PS312. We screened approximately 350 gametes (1300 homozygous F_2 lines) in two mutagenic screens over a six-month period. We isolated homozygous F_2 single worm clones in 96-well plates each containing 40 µl OP50 solution per well and allowed the generation of enough dauers (modified wet-plate method for large-scale screenings). We screened for nictation depletion after approximately 14 days in 30 µl containing more than 300 dauers per well; the remaining 5–10 µl was used to recover the homozygous lines. Candidate strains were confirmed by multiple searches for the defective phenotype in at least three independent dauer inductions (with 6000–60 000 dauers per induction) using the wet-plate method described above.

(d) *Nictation and attachment calculations*

We counted the total number of dauers in nictation from $n = 1000$ dauers, and calculated means and standard errors from three to 12 independent replicates. Standard errors of the mean were corrected for small sample size ($n < 20$) [34]. We tested the normality of our data using the Shapiro–Wilk test.

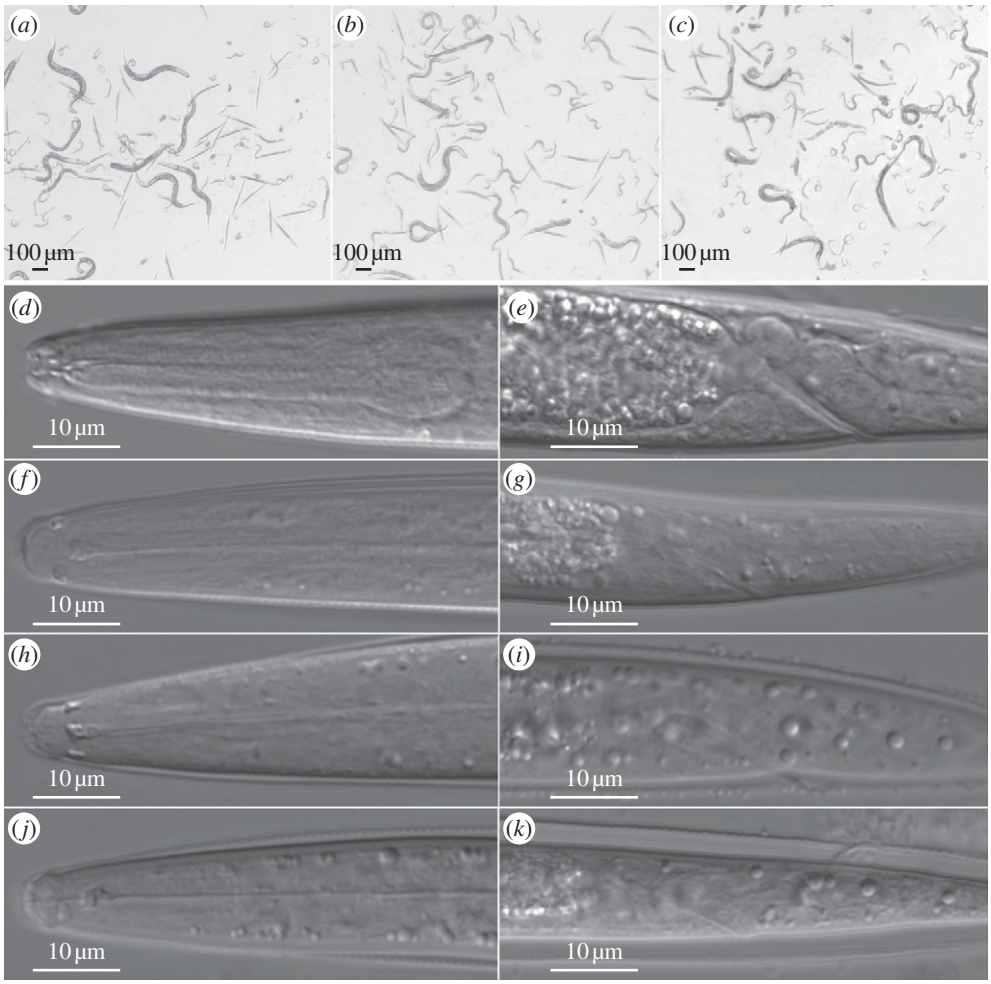

Figure 1. *Pristionchus pacificus* dauer juveniles after 15 days of induction in liquid culture: (*a*) PS312 strain, (*b*) *tu426* strain, and (*c*) *tu427* strain. Partial dauers are observed only in the nictation-defective mutants: (*b*) *tu426* and (*c*) *tu427*. A dorsal view of the head region shows closed oral orifices and constricted pharynges of (*f*) PS312, (*h*) *tu426*, and (*j*) *tu427* dauer juveniles when compared with reference PS312 third juvenile stage (*d*). Lateral view of PS312 third juvenile stage with an open anus is shown (*e*). By contrast, lateral views of strains PS312 (*g*), *tu426* (*i*), *tu427* (*k*) display a closed anus of dauer juveniles. The dauer juvenile stage (*f*–*k*) shows higher amounts of lipid droplets throughout the body than the third juvenile stage (*d*,*e*).

We performed an ANOVA between the groups, and a Tukey's honestly significant difference (HSD) test to assess significant differences in nictation between the strains. To assess for differences in the time to dauer formation between *P. pacificus* PS312 and the mutant strains, we used a non-parametric Mann–Whitney *U*-test because some of the data did not follow a normal distribution.

To study the relationship between nictation and attachment, we used multiple replicates of *single* assays. We calculated the number of dauers in nictation on each plate immediately before host exposure and the number of dauers on the host 2 h later. We tested nictation and attachment to host relationship in two independent assays, with two replicates of 1000 dauers and three replicates of 5000 dauers, respectively. The correlation coefficient (r) and coefficient of determination (r^2) were calculated. ANOVAs and Tukey's HSD tests were also performed on these data.

3. RESULTS

(a) *Morphological characterization of* Pristionchus pacificus *dauer juveniles*

The dauer stage is an alternative developmental stage to the J3 stage of directly developing nematodes (figure 1). Under stressful conditions or high densities, *P. pacificus* juveniles enter the dauer stage (figure 1*a*). Specialized morphological and physiological characteristics, such as high lipid storage, strong and impermeable cuticle, no food intake and extended lifespan, among others, develop in the dauers for endurance (table 1). Several characteristics distinguish arrested dauer juveniles from the active J3 juveniles (table 1 and figure 1*d*–*k*). Specifically, *P. pacificus* (PS312) dauers possess thin bodies and a dark intestine, which indicates their non-functional gut (figure 1*f*,*g* and table 1). Dauers contain a constricted pharynx and a closed oral orifice with an internal plug (figure 1*f*,*h*,*j* and table 1) and closed anus (figure 1*g*). Dauers also show a specialized cuticle and a stereotypical gonadal arrest with fewer germ cells than J3 juveniles (not shown) as has been previously described for *C. elegans* (table 1) [47–49]. The mid-body region of dauers is characterized by a high density of lipid droplets, which is absent in the J3 stage.

(b) *Nictation behaviour in* Pristionchus pacificus *dauers and other nematode species*

We induced nictation behaviour in dauers by adding sand grains to the substrate of the worms. As previously described for other nematode species [8], *P. pacificus*

Table 1. Dauer characteristics in *P. pacificus*. Most of these characteristics have been described previously (see references); differences found in *C. elegans* are shown in bold.

category	dauer characteristic	references
(*a*) morphology	thin and dense body; axial ratio for *P. pacificus* is 16:1 (length:width), **for *C. elegans* it is 30:1**.	[35–37]
	remodelled foregut pharynx	[38]
	dark sealed intestine, generally darker than corresponding J3 stage, **or L2 for *C. elegans***	[35–37]
	closed mouth and constricted pharynx	[35–37,39]
	gonadal arrest	[35–37]
	strengthened, specialized cuticle with lateral ridges: peripheral ridges become more pronounced in *P. pacificus*, **whereas conspicuous lateral alae become visible for *C. elegans***	[35–37,39]
	fat bodies in intestinal and hypodermal cells	[40]
	remodelled neurons in *C. elegans*; has not been characterized for *P. pacificus*	[41]
(*b*) physiology	developmental arrest	[42]
	increase in lifespan reduced metabolic activity and dependence on internal energy storage; work in progress for *P. pacificus* (M. Mayer, A. Ogawa & R. Sommer, 2009, personal communication)	[37,43,44]
	resistance to environmental stress heat, cold, desiccation, oxidative stress and detergents such as SDS in *C. elegans*; has not been tested for *P. pacificus*	[35–37,43–46]
(*c*) behaviour	lethargic needle-like pose with reduced activity	[35–37]
	nictation	[35]

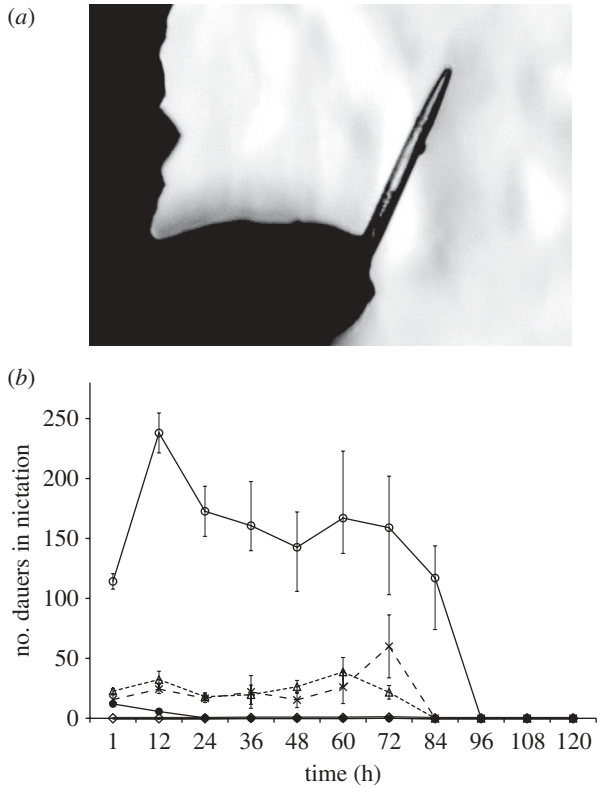

Figure 2. Nictation in *P. pacificus*. (*a*) A representative dauer in nictation. Its posterior end remains attached to the substrate, whereas its anterior stands erect with occasional waving. Note a lipid droplet often observed on the side of the pharynx region of dauers in nictation. (*b*) Number of dauers in nictation in populations ($n = 1000$) of two entomopathogenic *Steinernema* species (*S. feltiae* (dashed lines with triangles) and *S. carpocapsae* (dashed lines with crosses)), the slug parasitic *P. hermaphrodita* (double line with rhombi), and two *P. pacificus* strains (PS312 (solid lines with filled circles) and RS5386 (solid lines with open circles)). Error bars represent standard errors of the mean (s.e.m.) corrected for small sample size $n < 20$.

dauers lift all of the body off the substrate except the posterior tip. The nematodes keep a primarily straight posture (figure 2*a*) and occasionally wave their bodies in three-dimensional spirals and loops (electronic supplementary material, movie S1). Thus, *P. pacificus* dauer juveniles show a nictation behaviour typical of other nematodes.

We scored nictation and compared *P. pacificus* with other nematodes species (figure 2*b*). Initially (after 1 h), the free-living *P. pacificus* (PS312) exhibited nictation behaviours similar to that of the entomopathogenic nematodes *S. carpocapsae* and *S. feltiae*. Species began to exhibit differences after 12 h. *Pristionchus pacificus* PS312 started at around 15 dauers in nictation, and dropped to nearly zero after 24 h (figure 2*b*). The entomopathogenic *S. carpocapsae* and *S. feltiae* maintained 20–30 dauers in nictation until 36 h. Interestingly, *S. carpocapsae* reached a peak of nictation of around 60 dauers at 72 h, whereas *S. feltiae* reached a peak of around 40 dauers at 60 h (figure 2*b*). The slug parasitic *P. hermaphrodita* did not show any nictation throughout the whole period. By contrast, only the recently isolated *P. pacificus* strain RS5386 showed a higher proportion of nictation from the beginning and up to 96 h after-sand addition. Over 100 *P. pacificus* RS5386 dauers displayed nictation after 1 h, followed by a sharp increase at 12 h (over 200 dauers in nictation), followed by a drop to *ca* 150 dauers after 24 h, which persisted up to 4 days post-sand addition.

(c) *Nictation-defective* Pristionchus pacificus *mutant strains*

To test the hypothesis that nictation behaviour affects attachment abilities for host association, we used forward genetics to generate strains lacking this behaviour in the *P. pacificus* PS312 strain. In total, we isolated 11 nictation-defective mutants. Most of these mutant strains showed dauer entry defects, referred to as 'partial dauers' (table 2) [42,49,50]. To reduce the probability

Table 2. Resemblance of dauer characteristics in nictation-depleted *P. pacificus* mutants to reference PS312 dauers: 1, low; 2, medium; 3, high. Most dauer-like mutants (highest similarity index) are shown in bold.

dauer characteristics	***tu427***	*tu428*	*tu429*	*tu430*	*tu431*	*tu432*	*tu433*	***tu426***	*tu434*	*tu435*	*tu436*
dark body	**3**	1	3	3	2	2	3	**3**	1	3	2
thin body	**3**	1	3	2	2	2	2	**3**	2	2	2
constricted pharynx (does not pump)	**3**	1	3	3	3	3	3	**3**	2	3	3
thin dark intestine	**3**	1	3	2	3	3	2	**3**	2	2	2
lipid droplet accumulation	**3**	1	2	2	3	3	3	**3**	2	3	3
gonad arrest	**3**	1	2	2	3	3	2	**3**	2	3	2
absence of intermediate or partial dauers	**2**	1	2	1	1	1	1	**3**	1	1	1
similarity index (total)	**20**	7	18	15	17	17	16	**21**	12	17	15

that severe anatomical defects are the cause of nictation-defective phenotypes, we compared the morphological characteristics of dauer juveniles between mutants and reference animals (table 2 and figure 1). We focused on conspicuous morphological characteristics (table 1) that define the dauer stage by assigning a similarity score to reference dauers, ranging from low to high (1–3) (table 2). We used the nictation-defective mutants with the highest similarity index to reference dauers for the rest of the study, i.e. *tu427* (score of 20) and *tu426* (score of 21). The nictation-defective mutants *tu426* (figure 1*b*,*h*,*i*) and *tu427* (figure 1*c*,*j*,*k*) showed all dauer characteristics described for *P. pacificus* PS312 (figure 1*a*,*f*,*g* and electronic supplementary material, tables S1 and S2). Specifically, the oral orifices are closed and contain an internal plug [48] and their pharynges are constricted (figure 1*f*,*h*,*j*) [49]. In all three strains, the intestine is reduced and the mid-body region is characterized by a high density of lipid droplets. In figure 1*g*,*i*,*k*, the closed anus of the dauers is shown.

We observed an asynchrony in dauer formation between the reference and nictation-defective mutant strains (electronic supplementary material, figure S1). *Pristionchus pacificus* PS312 developed dauer juveniles approximately 120 h after eggs laying begins, whereas *tu427* mutant animals developed dauer juveniles after 125 h. The *tu426* mutant animals were significantly slower (Mann–Whitney test, $U = 2967.5$, *tu426* $n = 53$, PS312 $n = 56$, $p < 0.001$ two-tailed) in developing dauers; they spent more than 200 h in liquid culture before dauers appeared (electronic supplementary material, figure S1). Age differences observed in dauer formation between the different strains have not been shown to affect nictation behaviour. Previous observations in our laboratory comparing same dauer strains 12 or 25 days after induction showed no relevant differences in nictation numbers (data not shown).

(d) *Relationship between nictation in* Pristionchus pacificus *strains and attachment to the laboratory host* Galleria mellonella

To resolve the role of nictation in *P. pacificus*, we measured nictation rates of different strains and studied their attachment abilities to the larva of the moth *G. mellonella* (figures 3 and 4). The two mutant strains, *tu427* and *tu426*, show a complete absence of nictation during the nictation induction assays (figures 3 and 4). By contrast, one recent isolate from la Réunion island, which was

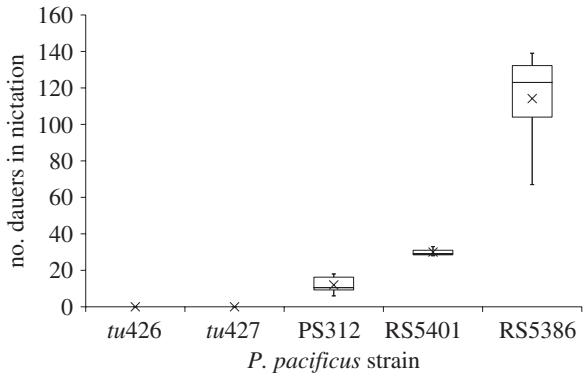

Figure 3. Number of dauers in nictation in populations ($n = 1000$) of *P. pacificus* nictation-defective mutant strains *tu426* and *tu427*, the reference laboratory strain PS312 and two strains from la Réunion island: RS5401 and RS5386. Maximum and minimum values are shown by whiskers, upper (75%) and lower (25%) quartiles are shown by the upper and lower limits of each box, medians and means are shown by the straight line and cross within each box, respectively.

kept in the laboratory for less than six months, showed a significantly higher nictation rate than the reference strain PS312 (ANOVA: $F = 142.17$, d.f. $= 4$, $p < 0.0001$; Tukey's HSD test: $p < 0.01$; electronic supplementary material, tables S1 and S3). RS5386 exhibits a two- to 10-fold higher nictation rate than the reference strain PS312 (figures 3 and 4). RS5401 showed a slightly higher nictation rate than PS312 (Tukey's HSD test: $p < 0.05$; table 3).

We found a proportional increase in the attachment to the host, which is directly related to the increase in nictation behaviour previously recorded for the different strains. The ability to attach to hosts increases dramatically when *P. pacificus* strains are able to nictate, as shown for reference and la Réunion strains (figure 4). In a sample containing 1000 dauers, we found absence of dauers attached to the hosts after 2 h of exposure for *tu427* and *tu426* (figure 4*a*). By contrast, we found approximately 20 *P. pacificus* PS312 dauer juveniles on host grubs after a similar exposure (figure 4*a*). The strains from la Réunion, RS5401 and RS5386, showed a relative increase in average attachment. However, despite the generally higher attachment of RS5401 to host grubs, this increase is not significantly higher than that of PS312 (ANOVA: $F = 106.25$, d.f. $= 4$, $p < 0.0001$; Tukey's HSD test: $p > 0.05$ (non-significant); figure 4*a* and

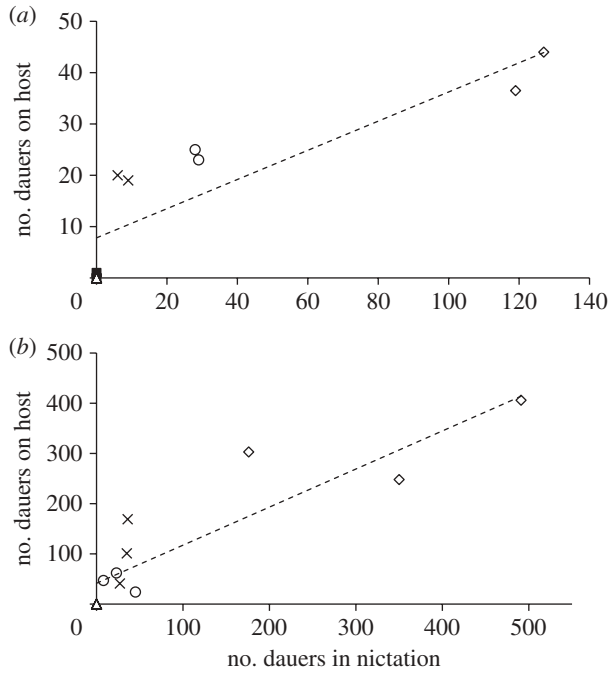

Figure 4. Relationship between nictation and attachment to the laboratory host *G. mellonella* for two nictation-defective mutant strains (*tu426* in solid squares, and *tu427* in open triangles), the reference laboratory strain (PS312 shown in crosses) and two recently isolated strains of la Réunion island (RS5401 in open circles, and RS5386 in open rhombi) in two different dauer concentrations assays: (*a*) total dauers on plate $n = 1000$. Correlation coefficient $r = 0.87$, and coefficient of determination $r^2 = 0.76$. (*b*) Total dauers on plate $n = 5000$. Correlation coefficient $r = 0.90$, and coefficient of determination $r^2 = 0.80$.

Table 3. Pairwise comparisons of nictation in *P. pacificus* strains using Tukey's honestly significant difference (HSD) test (cf. electronic supplementary material, table S1 and figure S3). n.s., non-significant differences.

strain A	strain B	*p*
tu426	*tu427*	n.s.
tu426	PS312	n.s.
tu426	RS5401	<0.01
tu426	RS5386	<0.01
tu427	PS312	n.s.
tu427	RS5401	<0.01
tu427	RS5386	<0.01
PS312	RS5401	<0.05
PS312	RS5386	<0.01
RS5401	RS5386	<0.01

electronic supplementary material, tables S2 and S4). By contrast, RS5386 showed nearly 40 worms attached to host and showed significantly higher numbers than the reference strain PS312, which only showed about 20 worms attached to the host (Tukey's HSD test: $p < 0.01$; figure 4*a* and electronic supplementary material, tables S2 and S4). The two nictation-defective mutant strains showed no significant difference in attachment ability when compared with each other (Tukey's HSD test: $p > 0.05$ (non-significant); figure 4*a* and table 4). In summary, a positive correlation ($r = 0.87$, $r^2 = 0.76$, $p \leq 0.001$) was found between nictation and attachment to hosts in *P. pacificus* strains.

To test whether population density had any effect on nictation or attachment to insect hosts, we repeated these experiments with a fivefold higher initial number of dauers ($n = 5000$ dauers) (figure 4*b* and electronic supplementary material, tables S2 and S4). We found that the relationship was maintained with relationship values that were almost identical ($r = 0.9$, $r^2 = 0.8$, $p < 0.001$). We conclude that density does not play a role in the positive relationship observed between nictation and host attachment. Taken together, we found a high positive correlation between the number of worms in nictation and the number of worms attached to host moth larvae. Coefficient of determination (r^2) and correlation coefficient (r) of nictation and attachment approach a value of 0.8 and 0.9, respectively, suggesting a relationship between both variables (figure 4). Thus, populations of nematodes with higher proportions of nictation are found to be more effective at attaching to mobile insects.

4. DISCUSSION

(a) *Use of mutant strains in combination with wild strains to study behavioural phenotypes in laboratory settings*

We took advantage of laboratory tools to combine, for the first time, mutagenesis-generated strains defective in a specific behaviour with recently isolated wild strains showing variable degrees of the specific behaviour. Classical forward genetic screens in *C. elegans* have proved to be a powerful tool for studying behaviours relevant to the species survival, such as mechanosensation mutants [51], chemosensation mutants [52], thermotaxis [53] and egg laying [54]. Populations of *P. pacificus* strains used in our study show a gradient in the proportions of nictation of each strain, which range from complete disruption of nictation in *tu427* and *tu426* mutants to high nictation in RS5386. Such a gradient of mutants, together with recently isolated strains, is required to correlate this behaviour to a particular function, such as host attachment. The low gamete number required to generate the nictation-defective strains *tu427* and *tu426* in our mutant screen shows that this behaviour may be complex and regulated by multiple genetic loci.

Nictation is absent in all stages of development, except in dauers; therefore programmes that act upstream of dauer formation, such as dauer induction or entry, may also affect behavioural phenotypes of the dauer. Presence of partial dauers in our nictation-defective mutants (table 2) suggests that nictation in some of our mutants may be affected owing to dauer formation defects. Previous studies in *C. elegans* of dauer formation mutants (daf), such as *daf-2*, have shown that these genes act independently on different aspects of development, such as entry and exit to dauer, intestinal pigmentation and reproduction [42]. Furthermore, previous studies have reported partial or intermediate dauer formation by mutations in *daf-9, daf-15, daf-16, daf-18, daf-20, daf-12* and unmapped *sy5315* X-linked mutation [49,55–58]. It remains to be tested whether any of these mutants also show defects in nictation behaviour. *Pristionchus pacificus* nictation-defective mutant strains that form partial dauers might contain mutations in the

Table 4. Pairwise comparisons of **nictation** (bold) and *attachment* (italics) results of *P. pacificus* strains at different densities (1000 and 5000 worms in single assays) using Tukey's HSD test (cf. electronic supplementary material, table S2 and figure S4). n.s., non-significant differences.

1000 dauers	*tu426*	*tu427*	PS312	RS5401	RS5386
tu426	—	*n.s.*	$p < 0.01$	$p < 0.01$	$p < 0.01$
tu427	**n.s.**	—	$p < 0.01$	$p < 0.01$	$p < 0.01$
PS312	**n.s.**	**n.s.**	—	n.s.	$p < 0.01$
RS5401	$\mathbf{p < 0.01}$	$\mathbf{p < 0.01}$	$\mathbf{p < 0.01}$	—	$p < 0.01$
RS5386	$\mathbf{p < 0.01}$	$\mathbf{p < 0.01}$	$\mathbf{p < 0.01}$	$\mathbf{p < 0.01}$	—
5000 dauers	*tu426*	*tu427*	PS312	RS5401	RS5386
tu426	—	—	—	—	—
tu427	—	—	n.s.	n.s.	$p < 0.01$
PS312	—	**n.s.**	—	n.s.	$p < 0.01$
RS5401	—	**n.s.**	**n.s.**	—	$p < 0.01$
RS5386	—	$\mathbf{p < 0.01}$	$\mathbf{p < 0.01}$	$\mathbf{p < 0.01}$	—

orthologues of any of the formerly mentioned genes. Further experiments are necessary to determine how genes that regulate dauer entry also affect behaviour.

In our study, we isolated and characterized mutants with a complete absence of nictation behaviour, but we did not screen for mutants with defects in specific individual steps and events of nictation. Nictation most probably consists of a number of individual events that are controlled by independent regulatory genetic units. Such regulatory units might in turn have different effects on host finding and/or attachment. Other behaviours in *C. elegans* have been observed to have different sensitivities. Mutant strains such as *unc-97* (uncoordinated) and *mec-3* (mechanosensation defective) showed different degrees of sensitivities in behavioural mechanosensory responses [58]; whereas male mating behaviour also contained multiple independent sub-behaviours controlled by different neuronal and genetic inputs [59,60]. For example, it is known that vulva location by the males is mediated by the neurons HOA and HOB, and that the genes *lov-1* and possibly *klp-6* and *pkd-2* mediate these responses [59,60]. Therefore, nictation may also be divided into sub-behaviours regulated independently as described for previous behaviours.

(b) *Nictation behaviour is relevant for host finding*

The evolution of parasitism involves a series of events, including an initial association with a host. Previous comparisons of nictating species and insect associations of different entomopathogenic nematodes suggested that nictation may provide a higher chance of contact with a host owing to a higher body surface area exposure to the transitory insect [9,13,61]. Comparative studies in *Caenorhabditis* species also suggested that nictation may be associated with the attachment of transitory animals [47,62]. However, few studies so far have investigated host finding or nictation behaviour in the diplogastrids [6,63], although these nematodes often show specific insect associations. *Pristionchus pacificus* is a necromenic nematode, i.e. uses its dead host as a source of food, and shows a life history tightly associated with beetles [19,20]. Previous comparisons of nictation in different nematode species suggested a relationship to host attachment, but the present study is the first to show a relationship of the evolutionary history of foraging behaviour in nematodes at the population level by the use of different *P. pacificus* strains.

Many aspects of nematode host finding are still unclear. Nematodes perceive their environment primarily by chemosensation, thermosensation and mechanosensation. Rhabditid nematodes are commonly described as 'cruisers' if they spend most of their time crawling and searching for resource-associated cues, such as insect host chemicals. The slug parasitic nematode *P. hermaphrodita* shows minimal nictation in our study, as has been previously reported [13], and may therefore apply a cruiser strategy. In *P. pacificus*, interception of the chemical communication system of the insect is likely to be involved in host preferences [64]. 'Ambushers' are instead more sedentary. It was initially assumed that ambush foragers were not as responsive to chemical cues as cruise foragers. However, it has since become apparent that they do respond to chemical cues, although their response is fundamentally different from cruise foragers [65]. *Steinernema* species, both cruiser and ambusher, respond strongly to volatile cues [66]. Our experiments show that *Pristionchus* species first have the ability to recognize and move towards host-associated volatiles by chemotaxis, which typically applies to a cruiser strategy [22,64]. Second, *P. pacificus* show nictation behaviour that applies to a typical ambusher behaviour as well. For other ambush foragers, stimuli from the environment have been demonstrated to be important for host finding [67], and environmental cues are used to assess patch quality [68,69] and select ambush sites [70,71]. Therefore, we propose that *P. pacificus* dauers may also have the ability to scan the surrounding environment, as shown for some *Steinernema* species [11]. We speculate that the differences in the variability observed within each strain may be a consequence of environmental differences across replicates and unidentified strain-specific traits related to host attachment, e.g. host sensing. It should be noted, however, that other differences between the genotypes/strains also affect these traits. Furthermore, we propose that nictation behaviour may also facilitate scanning and detecting host-associated cues by the dauer, such as volatile chemicals [12].

In conclusion, we provide evidence at the intraspecific level that nictation is associated with attachment. It is

tempting to speculate that nictation or nictation-like host finding behaviours are crucial during the initial steps of the evolution of parasitism. The specificity of this behaviour to the host-finding stage of nematodes, both in parasitic and non-parasitic species, reveals the relevance of nictation to understanding the origins of parasitism. Future studies should aim to understand the genetic and sensory regulation of this behaviour.

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