Identification of a conserved, orphan G-protein coupled receptor required for efficient pathogen clearance in *C. elegans*

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**Running title:** GPCR required for *C. elegans* pathogen clearance

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Abstract

G-protein coupled receptors contribute to host defense across the animal kingdom, transducing many signals involved in both vertebrate and invertebrate immune responses. Whilst it has become well established that the nematode worm Caenorhabditis elegans triggers innate immune responses following infection with numerous bacterial, fungal and viral pathogens, the mechanisms by which C. elegans recognises these pathogens have remained somewhat more elusive. C. elegans G-protein coupled receptors have been implicated in recognising pathogen-associated damage and activating downstream host immune responses. Here we identify and characterise a novel G-protein coupled receptor required to regulate the C. elegans response to infection with Microbacterium nematophilum. We show that this receptor, which we designate PCDR-1, is required for efficient pathogen clearance following infection. PCDR-1 acts upstream of multiple G-proteins including the C. elegans Goq ortholog EGL-30 in rectal epithelial cells to promote pathogen clearance via a novel mechanism.

Keywords

Caenorhabditis elegans, pathogen clearance, G-protein coupled receptor, Microbacterium nematophilum
**Introduction**

G-protein coupled receptors (GPCRs) are the largest, most diverse group of membrane receptors. They regulate a wide variety of physiological responses and consequently they are common drug targets for the treatment of many diseases (1). Signaling via GPCRs plays a critical role in the regulation of immunity and inflammation. GPCRs are expressed on the surface of many immune cells (2) and can be activated by host and bacterially-derived signals to regulate innate and adaptive immune responses (3). For example, the inflammatory mediator formyl-Met-Leu-Phe (fMLP) and the complement fragment C5a both bind to and activate GPCRs stimulating chemotaxis and production of inflammatory cytokines (4, 5). Given the therapeutic success of drugs which target GPCRs, the identification and characterization of GPCRs that regulate immune responses is likely to provide future therapeutic targets for the treatment of infectious and inflammatory diseases.

The role of GPCRs in regulating innate immune responses is conserved in invertebrates (6). Consequently, genetically-tractable invertebrate models can provide important insights into the role of GPCRs in host defense. Amongst these invertebrate models the nematode worm *Caenorhabditis elegans* (*C. elegans*) is extremely well suited to this task. The *C. elegans* genome is predicted to encode at least 1300 GPCR genes (7) accounting for approximately 7% of the protein coding genes in the *C. elegans* genome (8). Furthermore, infection of *C. elegans* with a variety of naturally-occurring and clinically-relevant pathogens, provided as a food source, regulates conserved signaling pathways that activate behavioural and cellular immune responses to protect the host (9). These pathways are known to play central roles in the host defenses of other species and represent important evolutionary-conserved components of innate immunity highlighting the utility of this model in understanding conserved innate immune responses.
Several GPCRs regulate *C. elegans* infection responses, demonstrating the conserved function of this receptor super-family in host defense. These include *dcar-1* (10), *fshr-1* (11), *dop-4* (12), *ser-1*, *ser-7* (13) and *octr-1* (14) which have all been implicated in the regulation of cellular immune responses, and *npr-1* which is required for avoidance of pathogenic bacteria (15).

We have previously shown that G-protein signaling is required for the *C. elegans* response to infection with the nematode-specific pathogen *Microbacterium nematophilum* (*M. nematophilum*) (16). *M. nematophilum* establishes a non-lethal, persistent infection in the rectum of *C. elegans*, triggering behavioural and cellular immune responses that include swelling around the rectal opening (the Deformed anal region (Dar) phenotype) (17), upregulation of defense genes (18) and avoidance of contaminated bacterial lawns (19). Animals with mutations in the *C. elegans* Gαq ortholog, EGL-30, fail to avoid contaminated bacterial lawns and do not trigger the Dar phenotype following infection (16). As a consequence, these animals become constipated and grow more slowly on *M. nematophilum* contaminated lawns (16). Furthermore, the *C. elegans* Gαo ortholog, GOA-1 acts antagonistically to EGL-30(Gαq) to suppress the Dar phenotype and limit pathogen clearance (13). The role of EGL-30(Gαq) and GOA-1(Gαo) in these responses suggests that GPCRs play an important role in recognizing and responding to the presence of *M. nematophilum*.

Here we identify a conserved, orphan GPCR, PCDR-1, required to promote the clearance of *M. nematophilum* infections from *C. elegans*. This is the first description of a phenotype associated with deletions in *pcdr-1*. We find that PCDR-1 is expressed in neurons, epithelial cells and vulval muscle and that it is required in rectal epithelial cells to regulate pathogen clearance. PCDR-1 acts upstream of, and in parallel to, EGL-30(Gαq) in this response. Our previous data indicate that the role of the Dar phenotype is to promote pathogen clearance (13); however, PCDR-1 appears to play a minor role in regulating the previously described cellular
and behavioural immune responses to *M. nematophilum* infection, suggesting that it regulates pathogen clearance via at least one novel mechanism.

**Results**

**PCDR-1 is a G-protein coupled receptor required for efficient pathogen clearance**

Infection of *C. elegans* with the naturally-occurring pathogen *M. nematophilum* triggers an immune response that includes swelling around the rectal opening known as the deformed anal region (Dar) phenotype (17). This response is associated with increased rates of pathogen clearance from the rectal opening (13) and requires signaling via the G-protein EGL-30(Gαq) (16). To identify GPCRs acting upstream of EGL-30(Gαq) in this response we screened viable GPCR deletion mutants obtained from the *Caenorhabditis* Genetic Centre (University of Minnesota) for the Dar phenotype and their ability to clear Syto13-labelled *M. nematophilum* infections (see materials and methods for screen details). Wild type animals were able to clear 50% of the Syto13 *M. nematophilum* within 90 minutes of transfer to plates without any bacteria (Figure 1C). We identified two strains containing deletions in the 6th and 7th transmembrane domains of the orphan GPCR F59D12.1; *f59d12.1*(gk1000) and *f59d12.1*(gk1122) (Figure 1A and B), that retained significantly more labelled pathogen than wild type animals after 90 minutes (Figure 1C). The ability of pathogen to attach to the cuticle was not altered in these strains since we observed similar steady-state levels of SYTO13-labelled *M. nematophilum* adhering to the rectum of wild type, *f59d12.1*(gk1122) and *f59d12.1*(gk1000) animals (Figure 1D). Given that this is the first phenotype to be associated with deletion in *f59d12.1* we designated the *f59d12.1* gene *pathogen clearance defective receptor-1* (*pcdr-1*).

Although both *pcdr-1* deletions were defective in their ability to clear labelled pathogen, we observed significant differences in the rate of pathogen clearance when comparing these two
deletions, with \( pcdr-1(gk1000) \) clearing pathogen significantly faster than \( pcdr-1(gk1122) \) (Figure 1C). To attempt to explain this difference, we sought to determine whether either of these deletions was a null allele of \( pcdr-1 \) using RTPCR. \( pcdr-1(gk1122) \) and \( pcdr-1(gk1000) \) are relatively small deletions in the 3’ end of \( pcdr-1 \) covering the 5\(^{th}\) and 6\(^{th}\) and 4\(^{th}\) and 5\(^{th}\) exons respectively (Figure 1A and 2A). As expected using primers covering these exons (Figure 2A) we failed to detect a PCR product in \( pcdr-1(gk1122) \) or \( pcdr-1(gk1000) \) (Figure 2B top 2 panels). However, using primers covering exons 1 and 2 of \( pcdr-1 \) (Figure 2A) we detected PCR products in both \( pcdr-1(gk1000) \) and \( pcdr-1(gk1122) \) (Figure 2B 3\(^{rd}\) panel) suggesting that neither of these alleles represents a null allele of \( pcdr-1 \). To determine whether \( pcdr-1(gk1122) \) and \( pcdr-1(gk1000) \) were loss-of-function alleles of the same gene we performed complementation tests. Pathogen clearance rates in animals heterozygous for either \( pcdr-1(gk1122) \) or \( pcdr-1(gk1000) \) were indistinguishable from wild type indicating that both deletions are recessive (Figure 1E). However, transheterozygote animals for the two \( pcdr-1 \) deletions failed to complement each other and retained significantly more pathogen than wild type animals after 90 minutes (Figure 1E) indicating that loss of \( pcdr-1 \) function is responsible for the pathogen clearance defect observed in these deletion strains.

To further confirm that the pathogen clearance defect observed in \( pcdr-1(gk1122) \) and \( pcdr-1(gk1000) \) strains was caused by \( pcdr-1 \) we performed rescue experiments. Several independent lines expressing a genomic fragment containing wild type \( pcdr-1 \) (fosmid WRM0618bH06) fully restored the ability of \( pcdr-1(gk1122) \) and \( pcdr-1(gk1000) \) animals to clear labelled pathogen (Figure 1F and G). Indeed, \( pcdr-1(gk1122) \) and \( pcdr-1(gk1000) \) animals expressing this genomic fragment cleared labelled pathogen significantly faster than wild type (Figure 1F and G).

**PCDR-1 is expressed in neurons, vulval muscle and rectal epithelial cells**
Where is PCDR-1 required to regulate pathogen clearance? The genomic rescuing fragment contained a 2.8Kb region upstream of the predicted pcdr-1 ATG (hereafter pcdr-1p); therefore, we sought to determine whether this sequence was sufficient to drive PCDR-1 expression in the cells where it was required for pathogen clearance by performing rescue experiments using the PCDR-1 cDNA under the control of this pcdr-1p. Expression of this transgene was sufficient to fully rescue the pathogen clearance defect of pcdr-1(gk1122) and pcdr-1(gk1000) (Figure 1F and G) suggesting that pcdr-1p drives expression of PCDR-1 in the cells where it is required for pathogen clearance. To identify these cells, we expressed GFP under the control of pcdr-1p. pcdr-1p∷GFP expression was observed in ciliated neurons in the head (Figure 3D-F) and neurons in the ventral nerve cord (VNC) (Figure 3A-C). In the VNC pcdr-1p∷GFP expression co-localised with acr-2p∷mCherry, which marks the cholinergic motor neurons, however we also observed GFP positive cell bodies which were not colocalised with this marker suggesting that PCDR-1 is expressed in both cholinergic and non-cholinergic neurons in the VNC (Figure 3C).

Interestingly, we also observed expression of pcdr-1p∷GFP in the vulval muscles (Figure 3G-I) and in non-neuronal cells in the tail (Figure 3K). To determine the identity of these non-neuronal cells we co-expressed mCherry from a 1.3Kb fragment of the egl-5 promoter that drives expression in rectal epithelial cells (20). pcdr-1p∷GFP expression co-localised with egl-5p∷mCherry (Figure 3L) indicating that PCDR-1 was expressed in the rectal epithelium.

The expression of several mammalian GPCRs is known to be regulated by infection (2). Since the expression pattern of PCDR-1 was determined in the absence of infection we next sought to determine whether expression of PCDR-1 was regulated during infection with M. nematophilum. Using qRTPCR we did not detect any significant differences in the expression of pcdr-1 between M. nematophilum-infected and uninfected wild type animals (Figure 3M). Furthermore, we did not observe any differences in the expression pattern of pcdr-1p∷GFP
following infection with *M. nematophilum* (R. McMullan, data not shown). This data suggests that expression of PCDR-1 in neurons, vulval muscle and/or rectal epithelial cells is sufficient to mediate pathogen clearance.

**PCDR-1 is required in rectal epithelial cells for efficient pathogen clearance**

To determine where PCDR-1 was required to mediate pathogen clearance we performed cell-specific rescue experiments using the PCDR-1 cDNA expressed from either the pan-neuronal *rab-3* promoter (*rab-3p, n::PCDR-1*) (21) or a 1.3Kb fragment of the *egl-5* promoter that drives GFP expression in the rectal epithelial cells B, U, F and K (*egl-5p, re::PCDR-1*) (20). Expression of PCDR-1 cDNA throughout the nervous system, using *rab-3p*, failed to rescue the *pcdr-1*(gk1000) pathogen clearance defect (Figure 4B). However, we observed some rescue of the *pcdr-1*(gk1122) pathogen clearance defect at 60 minutes although this rescue was no longer observed at 90 minutes (Figure 4A).

In contrast, expression of *pcdr-1* cDNA in rectal epithelial cells, using *egl-5p*, was sufficient to fully rescue *pcdr-1*(gk1122) (Figure 4A) and *pcdr-1*(gk1000) (Figure 4B) at all time points. These results demonstrate that, although neuronal PCDR-1 may play a minor role in pathogen clearance, the major site of action for PCDR-1 in this response to infection is the rectal epithelium.

**PCDR-1 acts upstream of, and in parallel to, EGL-30(Gαq) to mediate efficient pathogen clearance**

Previous studies have identified that EGL-30(Gαq) and LET-60(Ras) signaling pathways acting in the rectal epithelium trigger the Dar phenotype in response to infection (16). Activation of these pathways using gain-of-function *egl-30*(js126gf) or *let-60*(n1046gf) also results in increased pathogen clearance rates (R. McMullan, unpublished observations). To determine whether PCDR-1 acts via either of these pathways to regulate pathogen clearance we performed epistasis analysis using *pcdr-1*(gk1122) and gain-of-function *egl-30*(js126gf)
and let-60(n1046gf) alleles. Consistent with our previous observations, we observed a small decrease in the percentage of egl-30(js126gf) retaining labelled pathogen at 90 minutes when compared to wild type controls however, we were unable to observe an increase in pathogen clearance rates in let-60(n1046gf) (Figure 5). Furthermore, the percentage of pcdr-1(gk1122);let-60(n1046gf) animals retaining labelled pathogen was not significantly different from pcdr-1(gk1122) animals alone (Figure 5), suggesting that PCDR-1 does not signal via LET-60(Ras) to regulate pathogen clearance. Conversely, pcdr-1(gk1122);egl-30(js126gf) animals retained significantly more labelled pathogen than pcdr-1(gk1122) alone. However, these animals still retained more labelled pathogen than either egl-30(js126gf) alone or wild type controls (Figure 5). Taken together, these results suggest that PCDR-1 regulates pathogen clearance via at least two downstream pathways, one of which requires on EGL-30(Gαq).

PCDR-1 has a minor effect on the known cellular immune responses to M. nematophilum infection

How does PCDR-1 mediate efficient pathogen clearance? When C. elegans is exposed to M. nematophilum-contaminated bacterial lawns it triggers protective cellular immune responses, including induction of defense genes (18) and the Dar phenotype (17). In addition, changes in C. elegans behaviour following infection result in avoidance of contaminated lawns (19). Although the relationship between these responses and pathogen clearance has not been fully established, our previous data indicate that one role of the Dar phenotype is to promote pathogen clearance (13). Therefore, we sought to determine whether changes in cellular immune responses or behavioural avoidance of the pathogen could account for the failure of pcdr-1(gk1122) and pcdr-1(gk1000) to efficiently clear pathogen infections.

The Dar phenotype is associated with increased rates of pathogen clearance from the rectal opening (13). Mutations in genes that are required for the Dar phenotype, including unc-73(ce362) and mpk-1(kul), result in an almost complete failure to trigger the Dar phenotype
following infection (16) and decreased rates of pathogen clearance (R. McMullan unpublished observation). Therefore, we scored the Dar phenotype of \( \text{pcdr-1}(gk1122) \) and \( \text{pcdr-1}(gk1000) \) animals following infection. In contrast to \( \text{unc-73}(ce362) \) and \( \text{mpk-1}(ku1) \) animals where the Dar phenotype is almost completely absent following infection, we only observed small differences in the Dar phenotype of \( \text{pcdr-1}(gk1122) \) or \( \text{pcdr-1}(gk1000) \) animals relative to wild type controls (Figure 6A). This suggests that failure to trigger the Dar phenotype in \( \text{pcdr-1}(gk1122) \) or \( \text{pcdr-1}(gk1000) \) does not fully account for the defective pathogen clearance observed in these animals.

The induction of host defense genes is also part of the cellular response to infection with \( M. \) nematophilum (18). Since \( \text{pcdr-1}(gk1122) \) and \( \text{pcdr-1}(gk1000) \) did not have a large effect on the Dar phenotype we sought to determine whether PCDR-1 could regulate this cellular response to infection by examining the expression of two host defense genes, \( \text{clec-60} \) and \( \text{f53a9.8} \), that are upregulated by infection with \( M. \) nematophilum (18). Using qRT-PCR we measured the induction of \( \text{clec-60} \) and \( \text{f53a9.8} \) following infection of wild type, \( \text{pcdr-1}(gk1122) \) and \( \text{pcdr-1}(gk1000) \) animals. Unlike previous studies, we were unable to detect a significant increase in \( \text{clec-60} \) expression following infection of wild type animals (data not shown). This mostly likely reflects differences in infection protocols as, in contrast to previous studies that collected RNA 6 hours after infection, we grew animals on infection plates for one generation. We observed a significant increase in \( \text{f53a9.8} \) expression following infection of wild type animals (Figure 6B and C). A similar increase was also observed in \( \text{pcdr-1}(gk1122) \) (Figure 6B) or \( \text{pcdr-1}(gk1000) \) (Figure 6C) animals.

**Changes in defecation are not responsible for PCDR-1 pathogen clearance defects**

Since \( \text{pcdr-1}(gk1122) \) and \( \text{pcdr-1}(gk1000) \) appear to only have a minor effect on the previously identified cellular responses to infection with \( M. \) nematophilum, we sought other explanations for the role of PCDR-1 in pathogen clearance. Although the role of defecation in mediating \( M. \) nematophilum...
nematophilum clearance has not been fully investigated, it seemed logical that it may be involved in removing the pathogen from the rectal opening. Therefore, we sought to determine whether the pcdr-1 pathogen clearance defect was caused by defects in the defecation cycle. C. elegans defecation occurs via a series of highly stereotyped muscle contractions that are initiated approximately every 50 seconds (22). To determine the length of each defecation cycle following infection, we measured the interval between one of these muscle contractions, the posterior muscle contraction, or pBoc. Wild type uninfected animals had a mean cycle length of 60 seconds and following infection this cycle interval was decreased to 47 seconds, indicating that infection increased defecation rate (Figure 7A). Uninfected pcdr-1(gk1122) and pcdr-1(gk1000) animals had a similar mean cycle interval to wild type animals and this was also decreased following infection (Figure 7A).

In wild type animals, the posterior body muscle contraction (pBoc) step of the defecation cycle is almost always followed by anterior body muscle contraction (aBoc) and then expulsion (Exp) (22). Whilst measuring the mean cycle length of infected animals we noticed that the Exp step was frequently absent. The percentage of complete cycles (those in which Exp followed pBoc) was decreased from 98% in uninfected wild type animals to 38% following infection (Figure 7B). A similar decrease was also observed in pcdr-1(gk1122) and pcdr-1(gk1000) (Figure 7B) suggesting that although infection is able to alter the C. elegans defecation cycle, this is not regulated by PCDR-1.

Defective pathogen avoidance in PCDR-1 does not fully account for pathogen clearance defects

Infection with M. nematophilum triggers changes in locomotion that lead to behavioural avoidance of the pathogen (16, 19, 23). Like the Dar phenotype this behavioural response requires EGL-30(Gaq) (16). Therefore, we sought to determine whether the pathogen clearance
defects we observed in strains carrying pcdr-1(gk1122) and pcdr-1(gk1000) deletions reflected decreased behavioural avoidance of the pathogen. To do this we first performed pathogen avoidance assays in which we scored the percentage of animals on and off lawns contaminated with M. nematophilum. 60% of wild type animals avoided M. nematophilum-contaminated lawns (Figure 8A). We observed a significant avoidance defect in pcdr-1(gk1000) and pcd-1(gk1122) animals with only 42% of pcd-1(gk1000) and 4% of pcd-1(gk1122) animals avoiding contaminated bacterial lawns (Figure 8A).

To determine whether deletions in pcd-1 were responsible for the pathogen avoidance defects we observed we made use of the genomic fragment that rescued the pcd-1(gk1122) and pcd-1(gk1000) pathogen clearance defects (Figure 1F and G). Using these transgenes, we were unable to observe any rescue of the pcd-1(gk1122) pathogen avoidance phenotypes (Figure 8A). Therefore, we suggest that this strain contains an additional mutation that is responsible for this pathogen avoidance phenotype or that additional regulatory elements not present in our rescuing transgene are required for PCDR-1 to regulate pathogen avoidance. Regardless, the decreased pathogen clearance observed in pcd-1(gk1122) animals is not a consequence of their inability to avoid pathogenic lawns since this transgene was able to fully rescue the pcd-1(gk1122) pathogen clearance defect (Figure 1F and G). Conversely, the pcd-1(gk1000) pathogen avoidance defect could be fully rescued by expression of these transgenes (Figure 8A) suggesting that pathogen avoidance could contribute to the pathogen clearance defect in these animals.

To further investigate how pathogen avoidance contributes to differences in pathogen clearance rates we modified our infection assay by spreading M. nematophilum to the edges of the plate. In this ‘full plate’ assay animals were unable to avoid the pathogen. Wild type animals raised on ‘full plates’ were still able to clear labelled pathogen, although this occurred more slowly
than for animals raised on our standard assay plates (Figure 8B and C) suggesting that the ability to avoid pathogen lawns is important for efficient pathogen clearance. We observed a similar decrease in the rate of pathogen clearance in pcdr-1(gk1000) grown on ‘full plates’ when compared to pcdr-1(gk1000) animals raised on standard lawns (Figure 8C) suggesting that, although pcdr-1(gk1000) animals are partially defective in pathogen avoidance, under standard conditions this defect does not fully account for the observed pathogen clearance defect. In contrast, we did not observe any decrease in the pathogen clearance rates of pcdr-1(gk1122) mutants on standard vs ‘full plates’ (Figure 8B). This could be as a consequence of limitations in this assay since very few pcdr-1(gk1122) animals clear labelled pathogen under standard conditions, making it difficult to observe any further decreases in pathogen clearance rate.

Taken together, our results suggest that defective pathogen avoidance and a decrease in the Dar phenotype may contribute to the defective pathogen clearance we observed in pcdr-1(gk1000) animals. However, the failure to observe any large changes in the Dar phenotype and our inability to rescue the pathogen avoidance defect of pcdr-1(gk1122) suggests that PCDR-1 regulates pathogen clearance via multiple mechanisms, at least one of which is novel.

Discussion

Despite significant advances in understanding the immune responses that are activated by C. elegans during infection, a relatively small number of cell surface receptors that detect the presence of pathogens have been identified. These include the GPCRs DCAR-1 (10), FSHR-1 (11), DOP-4 (12), SER-1 and SER-7 (13) and OCTR-1 (24). Here we identify a previously uncharacterised GPCR required to regulate the host response to infection with M. nematophilum, adding PCDR-1 to the list of GPCRs required for host defense in C. elegans.
PCDR-1 is expressed in neurons, vulval muscle and rectal epithelial cells and our ability to rescue pcdr-1(gk1122) and pcdr-1(gk1000) by expressing the PCDR-1 cDNA from a rectal epithelial cell-specific promoter demonstrates that PCDR-1 is required in rectal epithelial cells for efficient pathogen clearance. The requirement for PCDR-1 in cells that mediate the cellular immune response to infection is consistent with previous observations using Drechmeria conispora (10) and Pseudomonas aeruginosa (11) where the GPCRs DCAR-1 and FSHR-1 act cell autonomously to regulate cellular immune responses. However, whilst expression of PCDR-1 in rectal epithelial cells was sufficient to regulate pathogen clearance, it should be noted that expression of PCDR-1 may be modified by additional regulatory elements upstream of the 2.8Kb region used for our rescue experiments and this expression may be required for additional functions of PCDR-1.

Signalling by EGL-30(Gαq) in rectal epithelial cells is both necessary and sufficient to trigger changes in cell morphology that cause the Dar phenotype (16) and the requirement for PCDR-1 in rectal epithelial cells raises the possibility that PCDR-1 exerts its effects on pathogen clearance via EGL-30(Gαq). Consistent with this, we find that PCDR-1 acts via EGL-30(Gαq) however two lines of evidence suggest that that multiple G-proteins act downstream of PCDR-1.

Firstly, epistasis analysis using animals carrying a gain-of-function egl-30(js126gf) allele demonstrates that, although egl-30(js126gf);pcdr-1(gk1122) animals clear infections more quickly than pcdr-1(gk1122) animals alone, pcdr-1(gk1122) is still able to decrease the pathogen clearance rates of egl-30(js126gf) animals. Secondly, whilst loss-of-function mutations in egl-30(ad805) result in a complete failure to trigger the Dar phenotype following infection with M. nematophilum (16) we only observed a small decrease in the Dar phenotype in pcdr-1(gk1000) and pcdr-1(gk1122) animals. Taken together our data suggests that PCDR-1 exerts its effects via EGL-30(Gαq) and an additional G-protein. The C. elegans genome
encodes 21 Go subunits including orthologs of each mammalian Go; Goq (EGL-30), Goα
(GOA-1), Goαs (GSA-1) and Goα12 (GPA-12) (25). We have previously demonstrated that
GOA-1(Goα) suppresses the Dar phenotype and limits pathogen clearance (13) suggesting that
GOA-1(Goα) does not act downstream of PCDR-1 to promote pathogen clearance however
the roles of the other G-proteins in regulating pathogen clearance and their relationship to
PCDR-1 remain to be established.

Although our data clearly demonstrate the PCDR-1 is required in the rectal epithelial cells for
efficient pathogen clearance, we also observed some rescue of pcdr-1(gk1122) when PCDR-1
was expressed in neurons, indicating a possible role for neuronal PCDR-1 in regulating
pathogen clearance. Further work is required to establish the role of neuronal PCDR-1 in
pathogen clearance, however several other C. elegans GPCRs act in neurons to regulate host
defences (11, 15, 24).

How does PCDR-1 signalling in rectal epithelial cells regulate pathogen clearance? Given our
previous observation that animals with decreased ability to trigger the Dar phenotype clear
infections more slowly (13) and the role of EGL-30(Goαq) in regulating the Dar phenotype (16)
one possible mechanism by which PCDR-1 could regulate pathogen clearance is via the
regulation of the Dar phenotype. However, we observed only a modest decrease in the Dar
phenotype of pcdr-1(gk1000) and pcdr-1(gk1122) suggesting that PCDR-1 is not the main
GPCR required to promote the Dar phenotype upstream of EGL-30(Goαq) and that PCDR-1
most likely regulates pathogen clearance via another mechanism. We also failed to observe any
differences in defecation or induction of antimicrobial peptides in pcdr-1 mutants, suggesting
that PCDR-1 does not regulate pathogen clearance by either of these mechanisms.

One possible explanation for the decrease in pathogen clearance rates could be an increase in
pathogen load caused by an inability to avoid pathogenic lawns. Although we observed a
pathogen avoidance defect in both pcdr-1(gk1122) and pcdr-1(gk1000) we propose that this
avoidance defect cannot fully explain the defective pathogen clearance in these animals for several reasons. Firstly, we have previously shown that an inability to avoid pathogen contaminated bacterial lawns increases pathogen load (23); however, we did not observe any increase in pathogen load in pcdr-1 mutants when compared to wild type controls. Secondly, pathogen clearance rates were further decreased when pcdr-1(gk1000) animals were infected under conditions where they were unable to avoid pathogen-contaminated bacterial lawns in a manner similar to wild type. Finally, we were unable to rescue the pathogen avoidance defect of pcdr-1(gk1122) using transgenes that were able to rescue the pathogen clearance defect in these animals. It should be noted that, whilst this final result demonstrates that pathogen avoidance cannot account for the pathogen avoidance defect in pcdr-1(gk1122), it does not fully exclude a role for PCDR-1 in the pathogen avoidance defect observed in pcdr-1(gk1122) animals. We did not test transgenes containing regulatory regions upstream of the 2.8Kb used to rescue the pathogen clearance defect of pcdr-1 and it therefore remains possible that regulation of PCDR-1 expression by additional upstream regulatory elements could be required for the ability of PCDR-1 to mediate pathogen avoidance. Taken together, our results suggest that although regulation of lawn avoidance and the Dar phenotype by PCDR-1 may play a role in promoting pathogen clearance PCDR-1 also acts via another mechanism. Genetic screens to identify further suppressor of pathogen clearance are likely to provide insight into this novel mechanism.

We have shown that PCDR-1 is required for efficient pathogen clearance; however, PCDR-1 is an orphan GPCR and the ligand that activates PCDR-1 to promote pathogen clearance remains to be identified. One possibility is that PCDR-1 acts as a pattern recognition receptor (PRR) to recognises a specific microbial product or pathogen associated molecular pattern (PAMP). Alternatively, PCDR-1 could act as a damage associated molecular pattern (DAMP) receptor recognising an endogenous signal released by *C. elegans* in response to infection. To
date no PRRs have been identified in *C. elegans* however DCAR-1 has been shown to act as a DAMP receptor and is activated by endogenous hydroxyphenyllactic acid (HPLA) which accumulates when worms are infected with the fungal pathogen *Drechmeria conispora* (10). The identification of the ligand for PCDR-1 will be key to further understanding its role host defense.

Receptors for biogenic amines including serotonin (13), octopamine (24) and dopamine (12) have all been shown to regulate immune function in *C. elegans* and PCDR-1 is a predicted ortholog of the *Drosophila* dopamine/ecdysone receptor DopEcR. This raises the possibility either PCDR-1 detects dopamine secreted by *M. nematophilum* or that infection induces the expression of endogenous *C. elegans* dopamine which acts as a DAMP to activate PCDR-1.

However, *cat-2* mutant animals that lack the ability to synthesise dopamine have wild type rates of pathogen clearance (R. McMullan, unpublished observation) suggesting that endogenously synthesised dopamine is unlikely to act as a DAMP following *M. nematophilum* infection.

By homology PCDR-1 is also predicted to have melatonin receptor activity (26, 27) suggesting that melatonin may act as a PAMP or DAMP to activate PCDR-1 during *M. nematophilum* infection. In support of this, expression of the *C. elegans* ortholog of arylalkylamine N-acetyltransferase (AA-NAT) (*anat-1*), which is required for synthesis of melatonin from serotonin, is upregulated following *M. nematophilum* infection (R. McMullan, unpublished observation). However, we were unable to demonstrate activation of PCDR-1 by melatonin biochemically *in vitro* (Isabel Beets, personal communication) and further experiments are required to determine whether PCDR-1 has melatonin receptor activity.

Human free fatty acid receptor 4 (FFAR4) and the hypocretin neuropeptide receptors HCRTR1 and HCRTR2 are all predicted orthologs of PCDR-1, suggesting that either neuropeptides or omega-3 fatty acids (ω3-FAs) could act as PCDR-1 ligands. A number of *C. elegans*
neuropeptides are regulated in response to infection (28) and it remains to be determined whether any of these acts as a DAMP during *M. nematophilum* infection. Similarly, one of the *C. elegans* genes required for synthesis of ω3-FAs (*fat-3*) is upregulated by infection with *Pseudomonas aeruginosa* and is required for basal immunity (29), although the role of ω3-FA in response to *M. nematophilum* remains unknown. Interestingly, DCAR-1 has recently been identified as a paralog of PCDR-1 and an ortholog of FFAR4, HCRTR1 and HCRTR2 raising the possibility that PCDR-1 and DCAR-1 may represent a family of related GPCRs required to recognise infection in *C. elegans*.

Although further experiments are required to identify a biological ligand for PCDR-1, immunomodulatory functions for FFAR4, hypocretin, dopamine and melatonin receptors have all been reported in mammals. FFAR4 has an anti-inflammatory role (30) while hypocretin, dopamine and melatonin receptors are express on cells associated with immune function (31-33) and have been reported to regulate phagocytosis in macrophages (34, 35). Therefore, it is possible that the function of PCDR-1 in host defense is conserved and further studies into its function in *C. elegans* may provide basic insights into evolutionary conserved roles of GPCRs in protecting animals from infection.

### Materials and methods

#### Strains

The following *C. elegans* strains were used in this study: N2, VC2258 (*pcdr-1(gk1122)*), VC2156 (*pcdr-1(gk1000)*). VC2258 and VC2156 were outcrossed twice to give RJM142 (*pcdr-1(gk1122)*<sup>2</sup>) and RJM149 (*pcdr-1(gk1000)*<sup>2</sup>). All strains were cultivated at 20°C on nematode-growth media (NGM) plates seeded with *E. coli* OP50, unless otherwise stated, and maintained as described previously (36).

#### Transgenes and germline transformation
Plasmids (listed as pRJM) were constructed using standard techniques and verified by sequencing. Transgenic strains (listed as impEx) were isolated by microinjection of the plasmid together with cc::GFP, egl-5p::mCherry (pRJM88) or acr-2p::mCherry (pSJN445) (a gift of S. Nurrish, Massachusetts General Hospital) at 50ng/μl as a marker. In all experiments matched animals not expressing the injection marker were assayed in parallel. Data was only included if the phenotype of non-transgenic animals was comparable to that of the parental strain.

**Fosmid transgenes**

The fosmid WRM0618bH06 containing 2.8Kb upstream of the predicted pcdr-1 ATG, the pcdr-1 coding sequence and 4 other genes (gpn-1, f59d12.2, C03H12.1 and ZK662.2) was injected at 20ng/μl into pcdr-1(gk1122). impEx57, 58, 59, 60 and 61 contain extrachromosomal versions of this fosmid. Rescue of the pathogen clearance defect was observed in all 5 lines. impEx57 was crossed into pcdr-1(gk1000).

**PCDR-1 transgenes**

2.8Kb upstream of the predicted pcdr-1 ATG was taken from the fosmid WRM0618bH06 and subcloned upstream of GFP to give pRJM185. This plasmid was injected at 20ng/μl. impEx52 contains an extrachromosomal version of pRJM185 with pSJN445. impEx53 and impEx54 contain extrachromosomal versions of pRJM185 with pRJM88. Similar expression of pRJM185 was observed in all 3 lines. The wild type pcdr-1 cDNA was obtained from yk616h8 (a gift of Yuji Kohara) and verified by sequencing. This cDNA was subcloned into pRJM185; to drive expression from the endogenous promoter (pRJM193), pSJN569; a vector driving expression from the rab-3 promoter that drives expression throughout the nervous system (21) (pRJM192), and pLG7; a vector driving expression from a 1.3Kb egl-5 promoter fragment that drives GFP expression in B, K, F, U, P12.pa and three body wall muscles in the posterior (20) (pRJM200). These plasmids were injected at 20ng/μl into pcdr-1(gk1122). impEx62 and impEx63 contain extrachromosomal versions of pRJM193. impEx59 contains an
extrachromosomal version of pRJM192. *impEx60* and *impEx61* contain extrachromosomal versions of pRJM200. *impEx59, impEx60* and *impEx62* were crossed into *pcdr-1*(gk1000).

*M. nematophilum* infection

Infection with *M. nematophilum* and scoring of the Dar phenotype was performed as described previously (13). Unless otherwise stated adult animals were transferred from OP50 plates to infection plates and F1 progeny were used for assays when they reached L4/adult stage. SYTO13 staining was performed as described previously (37). Following incubation with SYTO13 animals were either transferred to unseeded plates, for clearance assays as described below, or mounted for imaging. Experiments were performed in triplicate and repeated at least three times.

Lawn avoidance

Steady state lawn avoidance was determined by transferring 3 adult animals to standard NGM plates seeded with 200μl OP50, 10% avirulent *M. nematophilum* (UV336) or 10% *M. nematophilum* (CBX102) diluted in OP50. After 6 hours adult animals were removed and F1 progeny were allowed to develop at 20°C until they reached L4/adult stage. Animals were scored as on or off the bacterial lawn. Experiments were performed in triplicate and repeated at least three times.

Clearance of Syto13 labelled *M. nematophilum*

Animals were infected with *M. nematophilum* and SYTO13 labeling was performed as described previously (13, 37). Clearance assays were performed as described previously (13). Experiments were performed in triplicate and repeated at least three times.

GPCR mutant screening

A small-scale pilot screen of 54 GPCR deletion mutants available from the *Caenorhabditis* Genetics Center (University of Minnesota) was performed. The following strains were used: JT3 *aex-2(sa3)*, RB1423 *C49A9.7(ok1620)*, RB1837 *C54A12.2(ok2376)*, RB1321
These strains were infected with *M. nematophilum* and the Dar phenotype was scored as described above. Pathogen clearance rates were determined for a subset of the strains as described above. Experiments were performed in triplicate and strains with a significant change in either the Dar phenotype or the rate of pathogen clearance were identified for further study.

**Microscopy**

Quantification of SYTO13 staining was performed as described previously (13). Controls were imaged in parallel and experiments were performed on at least three separate occasions. At least 30 animals were imaged per condition.
For determination of the *pcdr-1* expression pattern, animals were washed from NGM plates in M9 buffer [22 mM KH$_2$PO$_4$, 42 mM Na$_2$HPO$_4$, 85 mM NaCl, 1 mM MgSO$_4$] and fixed in 4% paraformaldehyde. After washing with M9 animals were allowed to settle and a small volume was transferred to a glass bottom petri dish. Images were obtained using a Leica SP5 inverted microscope with 40x objective. Digital images were captured using Leica Confocal Software and processed to give maximum intensity projections of a Z-series using FIJI (NIH).

**RTPCR analysis**

Animals were grown on standard NGM plates seeded with *E. coli* strain OP50 and harvested in M9 buffer [22 mM KH$_2$PO$_4$, 42 mM Na$_2$HPO$_4$, 85 mM NaCl, 1 mM MgSO$_4$]. Samples were flash-frozen in liquid nitrogen and stored at -80 °C until use. Samples were homogenised using Lysing Matrix Y (MP Biomedicals) in a PowerLyser-24 Homogeniser (MO BIO Laboratories). RNA was extracted using the RNeasy Mini Kit (Qiagen) according to manufacturer’s instructions. cDNA synthesis and RT-PCR was performed using the One-Taq One-Step RT-PCR kit (New England Biolabs). Amplification of *act-1* cDNA was performed as a positive control. Primer sequences are listed in Table 1. Resulting amplicons were separated on a 2% agarose gel according to standard procedures.

**Quantitative RTPCR analysis**

Animals were infected with *M. nematophilum* as described above and total RNA was extracted using a RNeasy Mini Kit (Qiagen). qRTPCR analysis was performed by QStandard as follows; 500ng RNA was reverse transcribed using Qiagen quantitech reverse transcription according to manufacturer’s instructions. cDNA was amplified using Quantifast SYBR green mix (Qiagen) with each primer at a final concentration of 500nmol/L. Primer sequences are listed in Table 1. Copy numbers/reaction were derived from standard curves using Rotor-Gene software. Three reference genes; *cdc-42*, *pmp-3* and *Y54F10D.4* were used for normalisation. Experiments were performed in biological triplicate.
Defecation assays

Animals were infected as described in above. F1 progeny were scored when they reached the adult stage. Defecation assays were performed as described previously (38). At least 3 animals were scored for each condition. Experiments were performed at least three times.

Statistical analysis

In all cases statistical analysis was performed using Prism 6 (GraphPad Software). Normality was determined using a D’Agostino-Pearson omnibus normality test. Data was compared using a one-way ANOVA followed by Tukey HSP Post hoc multiple comparison test (for data with a Gaussian distribution) or Kruskal-Wallis test followed by Dunn’s multiple comparison test (for non-Gaussian data). Clearance assay data was compared using a two-way ANOVA followed by Tukey HSP Post hoc multiple comparison test. Statistical significance is presented in the text and on figures as follows; * P≤0.05, ** P≤0.01, *** P≤0.001, **** P≤0.0001, n.s. P>0.05.

Acknowledgments

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References


39. **Figure captions**

40. **Figure 1** PCDR-1 is a G-protein coupled receptor required for efficient pathogen clearance
A. The genomic structure of *pcdr-1*. *pcdr-1*(gk1000) and *pcdr-1*(gk1122) are partially overlapping deletions in the 3’ end of *pcdr-1*. The genomic region and *pcdr-1p* promoter used for rescue experiments are also indicated. B. The predicted protein sequence of PCDR-1. Predicted transmembrane domains are highlighted in grey. Regions deleted in *pcdr-1*(gk1000) are indicated by a dotted line. Regions deleted in *pcdr-1*(gk1122) are indicated by a solid line. Regions deleted in both *pcdr-1*(gk1000) and *pcdr-1*(gk1122) are indicated by a wavy line.

C. Animals were infected with *M. nematophilum* and pathogen attached to the rectal opening of adult animals was labelled with the nucleic acid stain SYTO13. SYTO13 labelled *M. nematophilum* was cleared from the rectal opening of wild type animals and 50% of animals remained colonized 90 minutes after transfer to assay plates (C). The clearance of labelled pathogen was significantly decreased in strains containing deletions in *pcdr-1* (*pcdr-1*(gk1122) and *pcdr-1*(gk1000)) (C). No significant differences in the amount of SYTO13 labelled *M. nematophilum* attached to the rectal opening were observed between strains (D). Pathogen clearance rates were significantly decreased in *pcdr-1*(gk1122)/(gk1000) transheterozygotes but not in animals heterozygous for either *pcdr-1*(gk1122) or *pcdr-1*(gk1000) (E). Rescue experiments were performed using the fosmid WRM0618bH06; which covers the coding region of *pcdr-1* and 2.8Kb upstream of the predicted *pcdr-1* ATG as well as 4 other downstream genes (see materials and methods for details). Expression of WRM0618bH06 in *pcdr-1*(gk1122) (F) and *pcdr-1*(gk1000) (G) rescued pathogen clearance defects. Indeed, rescued animals clear pathogen infections significantly faster than wild type animals (F and G). PCDR-1 cDNA, expressed under the control of the 2.8Kb region upstream of the predicted *pcdr-1* ATG (*pcdr-1p*), also rescued the pathogen clearance defects of *pcdr-1*(gk1122)(F) and *pcdr-1*(gk1000)(G) animals. n.s. indicates not significant * indicates significance relative to wild type. # indicates significance relative to *pcdr-1*(gk1122) (C and F) or *pcdr-1*(gk1000) (G) (see materials and methods for details of statistical analysis).
Figure 2. *pcdr-1*(gk1122) and *pcdr-1*(gk1000) are not null alleles

A. Schematic of PCDR-1 highlighting primers used for RTPCR and *pcdr-1*(gk1122) and *pcdr-1*(gk1000) deletions. Details of primers are given in Table 1. B. RTPCR for PCDR-1 (top 3 panels) in wild type, *pcdr-1*(gk1122) and *pcdr-1*(gk1000). NTC – no template control. ACT-1 (bottom panel) was used as a positive control.

Figure 3. PCDR-1 is expressed in neurons, vulval muscle and rectal epithelial cells

GFP was expressed under the control of the 2.8Kb region upstream of the predicted *pcdr-1* ATG (*pcdr-1p*) (B, E, H and K) in order to determine the cellular expression pattern of PCDR-1. This was injected into wild type animals with *acr-2p::mCherry* (A, D and G) or *egl-5p::mCherry* (J). We observed GFP expression in cholinergic (orange arrow) and non-cholinergic neurons (green arrow) in the ventral nerve cord (A-C), in several neurons in the head (D-F) and the vulval muscle (G-I). We also observed GFP expression in non-neuronal cells in the tail which co-localised with the *egl-5p::mCherry* rectal epithelial marker (J-L, the rectal opening is indicated with an arrow). qRTPCR was used to determine whether PCDR-1 expression was regulated by infection with *M. nematophilum*. No significant difference in PCDR-1 expression was observed between infected and uninfected wild type animals (M). n.s. indicates not significant

Figure 4. PCDR-1 is required in rectal epithelial cells for efficient pathogen clearance

To determine where PCDR-1 was required to mediate pathogen clearance we performed cell-specific rescue experiments expressing the PCDR-1 cDNA in neurons (using the pan-neuronal *rab-3* promoter, n::PCDR-1) or rectal epithelial cells (using a 1.3Kb fragment of the *egl-5* promoter, re::PCDR-1). Partial rescue of *pcdr-1*(gk1122) (A), but not *pcdr-1*(gk1000) (B), was
observed at 60 minutes when PCDR-1 was expressed neuronally. We were able to fully rescue
the pathogen clearance defect of pcdr-1(gk1122) (A) and pcdr-1(gk1000) (B) by expressing
PCDR-1 cDNA in the rectal epithelium. * indicates significance relative to wild type. #
indicates significance relative to pcdr-1(gk1122) (A) or pcdr-1(gk1000) (B) (see materials and
methods for details of statistical analysis).

**Figure 5.** PCDR-1 acts upstream of, and in parallel to, EGL-30(Gαq) to mediate efficient
pathogen clearance

Animals were infected with *M. nematophilum* and the percentage of animals retaining SYTO13
labelled pathogen was scored 90 minutes after transfer to unseeded NGM plates. The
percentage of animals retaining SYTO13-labelled pathogen was increased in pcdr-1(gk1122)
and slightly decreased in egl-30(js126gf) when compared to wild type controls. pcdr-
1(gk1122);egl-30(js126gf) animals retained significantly more SYTO13-labelled pathogen
than egl-30(js126gf) animals but significantly less than pcdr-1(gk1122) animals. The
percentage of pcdr-1(gk1122);let-60(n1046gf) animals retaining labelled pathogen was not
significant different from pcdr-1(gk1122). n.s. indicates not significant. See materials and
methods for details of statistical analysis.

**Figure 6.** PCDR-1 has a minor effect on cellular immune responses to *M. nematophilum*
infection

Adult wild type, pcdr-1(gk1122) and pcdr-1(gk1000) animals were infected as described in
materials and methods. The percentage of animals with the Dar phenotype was decreased very
slightly from 98% in wild type animals to 94.8% in pcdr-1(gk1122) and 84.9% in pcdr-
1(gk1000) (A).
To determine whether PCDR-1 could regulate expression of the antimicrobial peptide F53A9.8 we performed qRT-PCR of infected and uninfected wild type, pcdr-1(gk1122) and pcdr-1(gk1000) animals as described in the material and methods. Expression of F53A9.8 was increased following infection of wild type animals (B and C) and a similar increase was observed in pcdr-1(gk1122) (B) and pcdr-1(gk1000) animals (C). See materials and methods for details of statistical analysis. n.s. indicates not significant.

Figure 7. PCDR-1 does not regulate changes in defecation during infection

To determine whether a defect in defecation behaviour was responsible for the pathogen clearance defect of pcdr-1(gk1122) and pcdr-1(gk1000) we measured the defecation rate (as interval between pBoc contractions) (A). Infection with *M. nematophilum* decreased the interval between defecation cycles from 60sec to 47sec in wild type animals and a similar decrease was observed in pcdr-1(gk1122) and pcdr-1(gk1000) animals (A). In *C. elegans* defecation is achieved by a series of muscle contractions (pBoc, aBoc and Exp) that occur in a highly stereotyped manner (38). Following infection of wild type animals we observed that the final step in this cycle (The Exp or expulsion step) was frequently missing (B). This phenotype was also observed in pcdr-1(gk1122) and pcdr-1(gk1000) animals (B). n.s. indicates not significant * indicates significance relative to uninfected control. See materials and methods for details of statistical analysis.

Figure 8. Defective pathogen avoidance in PCDR-1 does not fully account for pathogen clearance defects

Pathogen avoidance was assessed by scoring the percentage of animals not on *M. nematophilum* contaminated lawns. 61% of wild type animals avoided contaminated lawns however both pcdr-1(gk1122) and pcdr-1(gk1000) were defective in this pathogen avoidance
response (A). Expression of WRM0618bH06 rescued the avoidance defect of pcdr-1(gk1000) but failed to rescue the pathogen avoidance defect of pcdr-1(gk1122) (A). To determine whether pathogen clearance rates were still decreased in pcdr-1(gk1000) and pcdr-1(gk1122) when they were unable to avoid pathogen-contaminated lawns we modified our infection assays by spreading bacteria to the edge of the plates (big lawn assay). Pathogen clearance rates were decreased in wild type animals in this big lawn assay (B and C) and we observed a similar decrease in pcdr-1(gk1000) animals (C). We also observed a small decrease in pathogen clearance rates of pcdr-1(gk1122) animals grown on big lawns although this was not significant (B). * indicates significance relative to wild type. # indicates significance relative to pcdr-1(gk1000) standard plates (A and C) (see materials and methods for details of statistical analysis).

Table 1 RT-PCR and qRT-PCR primers used in this study

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782
783
784
785
Anderson et. al._Figure 2
Anderson et al. Figure 3

acr-2p::mcherry
egl-5p::mcherry
pcdr-1p::gfp
pcdr-1p::gfp
merge
merge
control
M. nematophilum infected

Normalised copy number

Anderson et al. Figure 3
Anderson et al. Figure 4

A

Percentage of animals retaining labelled pathogen

Time (min)

B

Percentage of animals retaining labelled pathogen

Time (min)

pcdr-1(gk1122)

wild type

re::PCDR-1:: pcdr-1(gk1122)

n::PCDR-1:: pcdr-1(gk1122)

pcdr-1(gk1000)

wild type

re::PCDR-1:: pcdr-1(gk1000)

n::PCDR-1:: pcdr-1(gk1000)
Anderson et al._Figure 5

Percentage of animals retaining labelled pathogen at 90 min

Wild type
pcdr-1(gk1122)
egl-30(s126gf)
let-60(n1046gf)

**n.s.**

* *** **** ****

n.s. n.s.
**Figure 6**

A. Percentage of animals with the dash phenotype.

B. Normalised copy number.

C. Normalised copy number.

Anderson et. al. _Figure 6_
A

![Bar chart showing percentage of animals off lawn](image)

**Percentage of animals off lawn**

- wild type
- pcd-1(gk1122)
- genomic DNA:pcdr-1(gk1122)
- pcd-1(gk1000)
- genomic DNA:pcdr-1(gk1000)

B

![Line graph showing percentage of animals retaining labelled pathogen](image)

- pcd-1(gk1122) full plate
- pcd-1(gk1122)
- wild type full plate
- wild type

C

![Line graph showing percentage of animals retaining labelled pathogen](image)

- pcd-1(gk1000) full plate
- wild type full plate
- wild type
- pcd-1(gk1000)