Perspectives on programmed cell death in microbial communities: group selection, niche construction and phenotypic plasticity

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PCD was/is considered a hallmark of multicellularity.

Single cells in multicellular organisms commit 'suicide' for the benefit of the whole organism.

It was/is believed that PCD evolved in multicellular organisms via kin selection as an adaptation.
The phenomenon of PCD occurs in unicellular life

- Induced by environmental stresses like heat, UV light, nitrogen starvation, decrease in pH and antimitabolites
- Occurs in diverse unicellular eukaryote lineages
- Molecular biology knowledge variable depending on model organism
- Phenomenon detected in number of ways (usually imported from multicellular systems)

**Definition:** PCD is an “active, genetically controlled, cellular self-destruction driven by a series of complex biochemical events and specialized cellular machinery” (Berman-Frank et al., 2004)
Some ways to detect PCD

(Durand et al, 2011)
Phosphatidylserine externalisation

Unstained Annexin positive & Sytox positive

Sytox positive

Annexin positive

(Sathe et al, in review)
Why is there PCD in unicellular organisms?

The never-ending debate:
It is non-adaptive e.g. pleiotropic effect, autophagy during starvation
It is adaptive e.g. group level survival via preservation of resources or limitation of infection

Consider the levels of selection and evidence
1. Genic-selection? No, maybe, depends what you mean by ‘gene’
2. Cell level selection? No, except in 1 interpretation of PCD
3. Group level selection? Yes, lab evidence in 2 cases (Oh No!)
4. Kin selection? Yes, lab evidence, and also the usual explanation
5. Population level selection? Supportive field evidence only
6. Species level selection? I don’t know, possibly not
7. Clade level selection? No, and conceptually flawed
What is PCD in unicellular organisms?

Mechanistic definition: “PCD is ‘active, genetically controlled, cellular self-destruction driven by a series of complex biochemical events and specialized cellular machinery’” (Berman-Frank et al, 2004)

Differentiate what PCD fundamentally is from how it is realized.

<table>
<thead>
<tr>
<th>Types of death</th>
<th>Evolutionary definition</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCD</td>
<td>PCD is an adaptation to abiotic or biotic environmental stresses resulting in the death of the cell.</td>
<td>E. coli; C. reinhardtii; D. salina; D. discoideum; L. major</td>
</tr>
<tr>
<td>Ersatz PCD</td>
<td>Ersatz PCD is intrinsic to the cell but the trait itself has not been selected for death.</td>
<td>E. coli; D. viridis; D. tertiolecta</td>
</tr>
<tr>
<td>Incidental death</td>
<td>Incidental death is extrinsic to the cell.</td>
<td>Any organism</td>
</tr>
</tbody>
</table>
In pictures...

(A) Incidental death
(B) Ersatz PCD
(C) PCD: example 1
(D) PCD: example 2
(E) Multiple effects of death in mixed communities

(Durand and Ramsey, In press)
The ‘other’ (not kin selection theory) lenses through which we can view PCD in the unicellular world?

1. Group-level selection
Is PCD selected for at the level of the group?

2. Niche construction (and the ‘black queen hypothesis’)
Can PCD be selected for via a niche construction mechanism?

3. Phenotypic plasticity
Is the PCD trait phenotypically plastic?
If so, how is the plasticity selected for?
1. PCD and group selection

Is there evidence for group-level selection? I think so, yes. But others disagree e.g. \textit{Leishmania} example

- In populations of \textit{Escherichia coli}, PCD is an “altruism [that] can evolve, even when relatedness is low” (Refardt et al, 2013)
- In populations of \textit{Leishmania major} parasites “apoptotic promastigotes, in an altruistic way, enable the intracellular survival of the viable parasites” (van Zandbergen et al, 2006).
- PCD is ‘adaptive’ in \textit{Chlamydomonas, Dunaliella} etc.

\textit{Although distinguish between ‘adaptive’ and adaptation’}
PCD is required for group viability and reproduction in *Leishmania major* (van Zandbergen et al, 2006)
Death plays a role in transferring resources to others in *Dictyostelium sp.*

(Arnoult et al, 2001)
The Price formalism and PCD evolution

A general formulation of the Price equation (Luque, 2018)

\[ \bar{w} \Delta \bar{z} = \text{Cov}(w_i, z_i) \]  \hspace{1cm} (eq. 1)

\[ \text{Cov}(w_i, z_i) = \text{Cov}(W, Z) + E(\text{Cov}(w, z)) \]  \hspace{1cm} (eq. 2)

\[ \bar{w} \Delta \bar{z} = \text{Cov}(W, Z) + E(\text{Cov}(w, z)) \]  \hspace{1cm} (eq. 3)

Mean fitness and change in the PCD trait

Covariance between groups

Covariance between individuals in the group
2. Does PCD fulfil the criteria for niche construction?

The simplified criteria for niche construction (Laland et al, 2016)

**Criterion 1**

An organism must *significantly* modify environmental conditions

- Chlorophytes and dinoflagellates secrete infochemicals, allelopathic molecules
- These modify the environment to degree that impacts growth of other taxa as well as self
- Thus, the modification seems to be significant
2. Does PCD fulfil the criteria for niche construction?

**Criterion 2**
Organism-mediated environmental modifications must influence selection pressures on a recipient organism

- The environmental changes impact fitness of self and others.
“How an organism dies affects the fitness of others” (Orellana et al, 2013)

(Durand et al, 2014)
2. Does PCD fulfil the criteria for niche construction?

**Criterion 3**
There must be an evolutionary response in at least one recipient population caused by the environmental modification

- There is some (limited) evidence that PCD is selected *for* (not just selection *of* the trait).
- PCD is adaptive, but evidence for a historical evolutionary response is limited.
- The evolutionary response may have occurred in the actor (the taxon modifying the environment) and other taxa.
PCD outcompetes non-PCD

(Yordanova et al, 2013)

PCD renders others resistant to death

(Refardt et al, 2013)
3. Is the PCD trait phenotypically plastic?

Phenotypic plasticity is the change in the expressed phenotype of a genotype as a function of the environment (Scheiner, 1993)

The PCD example
- In a population there is more than just variation. The phenotype changes depending on the environmental stress

“PCD denotes a system that is probabilistic (the same input does not universally produce the same output), branching (some stages in the execution of the program can lead to a range of future states) and non-discrete (loss of viability can be transient or graded).

(Durand and Ramsey, In press)
3. Why is there phenotypic plasticity in the PCD trait?

1. Theoretical models of chlorophyte group formation “show by contrast that aggregations cannot form when competition is high” (referring to persistent aggregation and ALL cells remaining viable and metabolically active)

2. Simulations of the range of scenarios …… “under real parameter values for phytoplankton cells”

3. Stability brought about by metabolic inactivity or death

(Bouderbala et al, 2018)
Group formation

(A) Viable cells
(B) Dead cell
(C) Dividing cells

(Sathe and Durand, 2015)
FOR DISCUSSION

1. Are the explanatory frameworks of group selection, niche construction and phenotypic plasticity required, or even helpful, to explain PCD in the unicellular world?

2. Is the explanation for PCD adequately captured by kin selection, or do we need the other ‘lenses’?
Reproduction and propagation of artificially selected colonial yeast

(Ratcliff et al, 2012)
Cell death: major phenotypes

**Necrosis**
- Normal cell → Swelling → Disintegration

**PCD**
- Normal cell → Condensation → Apoptotic bodies

External insult

Genetically controlled
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<th>Markers of PCD</th>
<th>Interpretations</th>
<th>Significance</th>
</tr>
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<tbody>
<tr>
<td>Transmission electron microscopy</td>
<td>Changes in ultrastructure can be characteristic of PCD</td>
<td>Gold standard</td>
</tr>
<tr>
<td>Definitive molecular characterizations</td>
<td>The genetic basis for PCD is established in some model systems</td>
<td>Gold standard</td>
</tr>
<tr>
<td>DNA laddering by gel electrophoresis</td>
<td>DNA laddering is the result of endonuclease activity, which is very specific for PCD</td>
<td>Hard sign</td>
</tr>
<tr>
<td>Ejection of the nucleus</td>
<td>Ejection of the nucleus is only found in programmed forms of death</td>
<td>Hard sign</td>
</tr>
<tr>
<td>Loss of membrane asymmetry</td>
<td>Loss of membrane asymmetry is specific for PCD but there remain questions concerning the assay used (annexin V)</td>
<td>Hard / Soft sign?</td>
</tr>
<tr>
<td>DNA (double or single strand) nicking</td>
<td>This form of DNA damage is non-specific and found in PCD and other conditions</td>
<td>Soft sign</td>
</tr>
<tr>
<td>Upregulation of PCD associated genes</td>
<td>Many PCD related genes are not specific for PCD and associated with other functions</td>
<td>Soft sign</td>
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<td>---------------------------------------------------</td>
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<tr>
<td>Caspase, caspase-like or metacaspase activity</td>
<td>These enzymes are required for most kinds of PCD, but may not be specific to PCD</td>
<td>Soft sign</td>
</tr>
<tr>
<td>Light microscopy</td>
<td>The cellular changes associated with PCD are not always visualized by light microscopy</td>
<td>Soft sign</td>
</tr>
<tr>
<td>Mitochondrial depolarization</td>
<td>This marker is typically positive during PCD, but it is not clear how specific it is</td>
<td>Soft sign</td>
</tr>
<tr>
<td>Increase in reactive oxygen species</td>
<td>ROS plays a role in most PCD mechanisms, but they are non-specific and associated with other stress responses</td>
<td>Soft sign</td>
</tr>
</tbody>
</table>

(Durand, in prep)
(Orellana et al., 2013)