Infection of *Coscinodiscus* spp. by the parasitoid nanoflagellate *Pirsonia diadema*: II. Selective infection behaviour for host species and individual host cells

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**Abstract.** The parasitoid nanoflagellate (PNF) *Pirsonia diadema* is host specific for the marine centric diatom *Coscinodiscus* spp. Experiments showed that flagellates significantly prefer *C. wailesii* over *C. granii* as host species (interspecific selectivity). This preference was independent of light conditions (dark, irradiance of 10 and 70 μmol m⁻² s⁻¹) and temperature (10 and 15°C). Among unicellular host diatoms, the infection behaviour was selective for individual cells: already infected *C. granii* cells were more attractive for further flagellate attachment than non-infected cells (intraspecific selectivity). Individual cells (~1%) remained uninfected for some time. *Coscinodiscus granii* cultures kept in darkness were more rapidly infected than those kept in the light. It is suggested that photosynthesis enhances the formation of individual physiological properties of diatoms leading to intraspecific variability of susceptibility.

**Introduction**

Field studies demonstrated that in the North Sea many common and bloom-forming planktonic diatoms are infected by a variety of host-specific parasitoid protists of various taxonomic groups. Many parasitoid protists infect only one diatom species, whereas others infect several host species (e.g. Zuelzer, 1927; Drebes, 1968, 1984; Schnepf, 1994; Drebes et al., 1996; Kühn, 1996/97, 1997a; Kühn et al., 1996; Schweikert and Schnepf, 1996). *Pirsonia diadema* is a parasitoid nanoflagellate (PNF) that infects only *Coscinodiscus granii*, *Coscinodiscus wailesii* and *Coscinodiscus concinnus* (Kühn et al., 1996). The colourless flagellates attach to the diatom frustule. A thin pseudopodium pierces the rimoportulae (tubular passages through the siliceous diatom cell wall). Inside the silica shell it forms the 'trophosome' where host cytoplasm is phagocytized and digested. The 'auxosome' remains outside the frustule, grows and divides several times to form up to 30 flagellated infective offspring. The taxonomic position of *Pirsonia* is still unknown. Electron microscope investigations on the ultrastructure, however, suggest that it belongs to the Straminipiles (Schweikert and Schnepf, 1997).

Studies on the interactions between *P. diadema* and *C. granii* showed that spreading of infections strongly depended on the internal biological behaviour of the flagellate, e.g. chemosensory response, swimming patterns and age of flagellate (Kühn, 1997b). Light microscope observations and preliminary experiments indicated that *C. wailesii* was the preferred host species, whereas *C. concinnus* was least attractive as a host (interspecific selectivity). It was not clear whether this was caused by differences in morphological and/or physiological properties between the diatom species. Among unialgal cultures of *C. granii* and *C. wailesii*, certain individual cells were apparently more easily infected than others (intraspecific selectivity). Studies on parasitoid behaviour are rare, however, and...
to the author's knowledge both inter- and intraspecific selective infection behaviour of parasitoid protists has not yet been investigated.

Observations had indicated that *C.granii* cells were more rapidly infected when maintained in darkness. This contrasted with reports of reduced infectivity of freshwater chytrid zoospores (Canter and Jaworsky, 1981), the marine phycomycte *Ectrogella* (Raghukumar, 1978) and the parasitoid dinoflagellate *Paulsenella* (Schnepf and Drebes, 1986) when kept in the dark.

It has been suggested that nutrient- or light-deficient algal populations are more susceptible to fungal infections (Canter and Lund, 1969; Reynolds, 1984), but studies on chytrid infections of freshwater diatoms had shown that phosphorus-limited host cells were less susceptible to infections than non-limited host cells (Bruning, 1991b). Since light-deficient *Coscinodiscus* cultures were more rapidly infected by *P.diadema*, it appeared that in this case the hypothesis of increased susceptibility under unfavourable growth conditions might hold true. Experiments were carried out to investigate the (i) interspecific and (ii) intraspecific infection behaviour of the parasitoids.

**Method**

The isolation and cultivation of *P.diadema* and *Coscinodiscus* spp. have been described by Kühn (1997b) and will be mentioned only briefly here. Both organisms were isolated from plankton samples collected in the German Bight, North Sea. Stock cultures of flagellates were maintained in small plastic Petri dishes with C.granii as host diatom and modified f/2 medium (Guillard and Ryther, 1962) at 15°C. Twice a week, flagellates and infected cells were transferred to a new host culture. Stock and experimental cultures were placed on shelves illuminated from above with white fluorescent light (Osram LS8/19, Daylight 5000 Deluxe) on an 18 h light:6 h dark cycle. Irradiance was controlled by varying the number of fluorescent bulbs or the light bank and/or placing one or more layers of neutral-density filter between culture compartments and the light source. Light intensities were measured with a Li-Cor integrator (Model LI-185 B) and a spherical quantum sensor (Model SR.NO.). Since no good fixative for preserving *Pirsonia* was found, living samples were counted in Petri dishes under a stereomicroscope.

**Experiment 1 (interspecific selectivity: effects of light and temperature)**

In order to test the selectivity of *P.diadema* for different diatom species, *C.granii* and *C.wailesii* were chosen as host diatom species. To 'amplify' the expected preference for *C.wailesii*, a mixed experimental culture of *C.wailesii* with a very small diameter (120-125 μm) and low density (30 cells ml⁻¹) was used. The *C.granii* culture contained cells twice as large (240-250 μm) with a 2.5-fold density (75 cells ml⁻¹). Experiments were carried out in 300 ml Erlenmeyer flasks containing 200 ml of the mixed diatom culture (one stock culture was divided into subsamples) which had just reached the stationary phase. A culture in the stationary phase was chosen to minimize physiological differences between treatments due to possibly different physiological properties of diatoms during growth.
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order to test whether selectivity was influenced by light and/or temperature, four different treatments were applied: treatment (a), 10°C and 70 μmol m⁻² s⁻¹; treatment (b), 15°C and 70 μmol m⁻² s⁻¹; treatment (c), 15°C and 10 μmol m⁻² s⁻¹; treatment (d), 15°C and darkness.

Stock cultures had been grown at 15°C and 10 μmol m⁻² s⁻¹, and experimental cultures were pre-adapted to changed conditions for 1 day. As inoculum for infection, 0.5 ml of a P. diadema-infected C. granii culture (containing both flagellates and infected diatoms) was used. On the day after inoculation (Day 1), each flask was gently mixed and subsamples were taken. Sampling was repeated daily until all diatoms were infected. Pirsonia diadema in the inoculum was not pre-adapted to 10°C (for all treatments: four replicates, five subsamples; n = 20; error bars = 95% confidence level).

Experiment 2 (intraspecific selectivity)
Observation had shown that P. diadema flagellates disperse homogeneously in suspension when no diatoms are present. When the flagellates were added to unialgal cultures of host diatoms, the selectivity for individual cells seemed to decrease with increased preference for the species (intraspecific selectivity highest among C. concinnus, lowest among C. wailesii). While infected cells seemed to be most attractive for further infections, some individual diatoms remained uninfected for a very long time. As infections spread faster among C. wailesii than C. granii, the latter was chosen as the food diatom for the experiments.

(a) Succession of infection stages among host cells. In C. granii cultures infected by P. diadema, a succession of infected stages of host cells could always be observed. In order to describe this development of infected stages, three replicates of 9 ml C. granii suspension with an initial density of 15 cells ml⁻¹ were inoculated on Day 0 with one C. wailesii cell infected by one P. diadema flagellate. Experiments were carried out in Petri dishes under lighted conditions. The number of infected diatom stages was determined daily as a percentage of the total number of diatoms. Five different stages of infected diatoms, according to the number of infecting P. diadema flagellates per valve, were distinguished: 0, no flagellates attached; I, 1–10 flagellates; II, 11–100 flagellates; III, up to 400 flagellates, as offspring had already developed; IV, empty frustules resulting from infections. The mean values of three replicates are given in the Results.

(b) Effects of light/darkness. From the previous experiment (succession of infection stages of C. granii), it became obvious that it was very time consuming to distinguish between five different stages of infected diatoms. Because counting of living samples limited the number of replicates, the experiment only distinguished between non-infected and infected cells in the following experiment in order to obtain enough data that could be treated statistically; succession of infected diatom stages was observed to be comparable to that in Experiment 2a.

As it was not clear which factors might affect susceptibility or temporary resistance of C. granii to infections by P. diadema, six treatments with different
Table 1. Description of treatments investigating intraspecific selectivity of *P. diadema* for individual *C. granii* cells (Experiment 2b)

<table>
<thead>
<tr>
<th>Treatment 1</th>
<th>Treatment 2</th>
<th>Treatment 3</th>
<th>Treatment 4</th>
<th>Treatment 5</th>
<th>Treatment 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diatom density (cells ml⁻¹)</td>
<td>110</td>
<td>110</td>
<td>170</td>
<td>170</td>
<td>170</td>
</tr>
<tr>
<td>History of cultivation prior to experiment</td>
<td>3 days in total darkness, log phase</td>
<td>3 days in total darkness, log phase</td>
<td>Light, end of log phase</td>
<td>Light, end of log phase</td>
<td>Light, for 2 days in stationary phase</td>
</tr>
<tr>
<td>Infected cultures were maintained/ transferred to</td>
<td>Dark</td>
<td>Light</td>
<td>Dark</td>
<td>Light</td>
<td>Dark</td>
</tr>
</tbody>
</table>

light/dark conditions and logarithmic/stationary growth phases as parameters were tested (see Table 1). For the experiments, 50 ml of diatom cultures were transferred into 70 ml disposable culture tissue flasks (DCTF) and infected with 20 µl *P. diadema* flagellate suspension (without host cells). For counting, those diatoms that were infected by only one flagellate (single infection) were distinguished from those infected by several flagellates (multiple infection), although the number of infecting flagellates was not determined in multiple infections. Sampling started 1 day after inoculation (Day 1) and was repeated daily until the number of non-infected diatoms had declined too far for maximum infection rates on following days (six replicates, four subsamples; *n* = 24; error bars = 95% confidence level).

**Results**

**Experiment 1: Interspecific selectivity**

In mixed cultures of *C. wailesii* and *C. granii*, *C. wailesii* cells were infected more rapidly by *P. diadema* than *C. granii*, although present at lower density and being of smaller cell sizes (Figure 1a–d). *Coscinodiscus granii* cells were infected after most of the *C. wailesii* cells had been infected. The number of non-infected diatoms did not increase during the experiment (cultures were in the stationary growth phase) so results are presented as a percentage of infected cells of *C. granii* (density 75 cells ml⁻¹) and *C. wailesii* (30 cells ml⁻¹). On Day 4 (treatment (a)), for example, in a mixed culture 0.6 cells ml⁻¹ *C. granii* were infected compared to 18.8 cells ml⁻¹ *C. wailesii*. At 15°C, all cells were infected within 4 days (treatment (b)), whereas 7 days were needed until 100% of the diatoms had been infected at 10°C (treatment (a)). At 15°C, no significant difference in infection was found between treatments with irradiance of 70 µmol m⁻² s⁻¹ (treatment (b)) and 10 µmol m⁻² s⁻¹ (treatment (c)). Infection among *C. granii* spread much faster in the cultures that were maintained in the dark (treatment (d)) than in those which had been exposed to light (treatments (b) and (c)). To prove the same for *C. wailesii* would have required a unialgal culture with a higher density.

The results of this experiment are in accordance with preliminary light microscope observations indicating that in mixed cultures *P. diadema* flagellates
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Fig. 1. Percentage of cells infected by *P. diadema* in a mixed culture of *C. grani* (white bars) and *C. wailesii* (shaded bars) at different irradiances and temperatures (Experiment 1; see the text). Diatom cultures were in the stationary growth phase; percentage values relate to different densities of *C. grani* (75 cells ml⁻¹) and *C. wailesii* (30 cells ml⁻¹) (error bars: mean ± 95% confidence limit; n = 20).

Congregated more rapidly around *C. wailesii* than *C. grani* cells. It should be noted that starving *P. diadema* often succeeded in infecting *C. wailesii*, but not *C. grani*.

**Experiment 2: Intraspecific selectivity**

(a) Succession of infection stages among diatoms grown under light conditions. In *C. grani* cultures infected by *P. diadema*, the diatoms were not equally infected by flagellates, but there was a succession of stages of infection among diatoms. Infection started to spread ~2 days after inoculation, and on Day 3 the first empty frustules of infected cells were found (Figure 2). Although on Day 4 the number of infective flagellates was several orders of magnitude higher than that of diatoms, so that all cells could have been multiply infected, ~1% of the *C. grani* still remained uninfected. These cells were not resistant, however, but were also infected the following day. This succession of infection stages indicated that *C. grani* grown under light conditions were individually different in their attractiveness for infection by the parasitoids.
Fig. 2. Succession of infection stages among *C. granii* cells infected by *P. diadema* (Experiment 2a). Number of attached flagellates per valve: 0, no flagellates attached; I, 0–10 flagellates; II, 11–100 flagellates; III, up to 400 flagellates (attached flagellates were dividing); IV, empty frustules due to infection.

(b) Effects of light/darkness. *Coscinodiscus granii* cells changed their appearance according to the light conditions and growth phase: *C. granii* cells placed in continuous darkness for 3 days were slightly paler than those grown under light conditions, but appeared healthy and resumed growth within 1 day when transferred back into light. Diatoms in the stationary phase became deeply pigmented and appeared more compact than those in the logarithmic growth phase. Cell division was impaired in the stationary phase, and the relationship of single cells (S) to pairs of not yet divided (‘double’) cells (D) changed in batch cultures from 2.3 S/D in the logarithmic phase to 0.4 S/D in the stationary phase, while the chlorophyll content per cell increased from ~300–320 pg cell\(^{-1}\) to 950 pg cell\(^{-1}\) (not shown; ‘double’ cells were counted as two cells).

In the experiments that investigated the intraspecific infection behaviour of *P. diadema* under different light conditions, the number of *C. granii* infected by *P. diadema* was higher in treatments that were either maintained in or transferred to total darkness (treatments 1, 3 and 5) than in those placed in light (treatments 2, 4 and 6) (Table II). In treatment 2, which had been maintained in the dark prior to inoculation with *P. diadema* and subsequently kept in the light, infection spread faster than in treatments which had been grown continuously under light conditions (treatments 4 and 6).

The experiment distinguished between single infections (one infecting *P. diadema* flagellate per diatom) as an estimation for only recently infected diatoms, and multiply infected cells (diatom infected by several flagellates). On Day 1, in all treatments single infections prevailed over multiple infections by a factor of 2.5–9.2. On Day 2 and 3, this ratio decreased in all treatments to values below one, except in treatment 1. Again, in all treatments some *C. granii* cells remained temporarily uninfected. However, since the number of infective flagellates in the cultures exceeded that of healthy diatoms by several orders of
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Table II. Number of C.granii (cells ml⁻¹) infected by P.diadema in different treatments (Experiment 2b; see Table I). The experiment differentiated between cells that were infected with only one P.diadema flagellate (single infection) or several flagellates (multiple infection). Treatments 1, 3 and 5 were those that were kept in darkness. For details, see the text (mean ± 95% confidence level; n = 24).

<table>
<thead>
<tr>
<th></th>
<th>Treatment 1 (dark)</th>
<th>Treatment 2 (light)</th>
<th>Treatment 3 (dark)</th>
<th>Treatment 4 (light)</th>
<th>Treatment 5 (dark)</th>
<th>Treatment 6 (light)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3.07 ± 0.95</td>
<td>2.61 ± 0.72</td>
<td>1.49 ± 0.68</td>
<td>0.85 ± 0.42</td>
<td>0.71 ± 0.47</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Single</td>
<td>2.48 ± 0.78</td>
<td>2.25 ± 0.68</td>
<td>1.06 ± 0.55</td>
<td>0.71 ± 0.42</td>
<td>0.64 ± 0.41</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Multiple</td>
<td>0.60 ± 0.53</td>
<td>0.37 ± 0.26</td>
<td>0.43 ± 0.38</td>
<td>0.14 ± 0.20</td>
<td>0.07 ± 0.15</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Ratio s/m</td>
<td>4.1 ± 4.9</td>
<td>6.1 ± 6.1</td>
<td>2.5 ± 3.4</td>
<td>5.1 ± 10.2</td>
<td>9.2 ± 25.4</td>
<td>-</td>
</tr>
<tr>
<td>Day 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Total</td>
<td>29.24 ± 4.00</td>
<td>11.87 ± 1.28</td>
<td>7.58 ± 2.26</td>
<td>4.96 ± 1.65</td>
<td>7.23 ± 1.28</td>
<td>2.13 ± 1.04</td>
</tr>
<tr>
<td>Single</td>
<td>16.55 ± 1.18</td>
<td>3.53 ± 0.88</td>
<td>1.42 ± 0.69</td>
<td>1.84 ± 0.82</td>
<td>1.49 ± 0.65</td>
<td>0.71 ± 0.51</td>
</tr>
<tr>
<td>Multiple</td>
<td>13.97 ± 1.86</td>
<td>8.34 ± 1.33</td>
<td>6.16 ± 2.08</td>
<td>3.12 ± 1.28</td>
<td>5.74 ± 1.35</td>
<td>1.42 ± 0.69</td>
</tr>
<tr>
<td>Ratio s/m</td>
<td>1.18 ± 0.24</td>
<td>0.42 ± 0.17</td>
<td>0.23 ± 0.19</td>
<td>0.39 ± 0.50</td>
<td>0.26 ± 0.17</td>
<td>0.50 ± 0.06</td>
</tr>
<tr>
<td>Day 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1110</td>
<td>59.07 ± 3.42</td>
<td>74.16 ± 8.36</td>
<td>25.00 ± 2.99</td>
<td>67.75 ± 5.12</td>
<td>18.79 ± 4.64</td>
</tr>
<tr>
<td>Single</td>
<td>nd</td>
<td>6.77 ± 0.88</td>
<td>14.80 ± 2.13</td>
<td>5.95 ± 1.40</td>
<td>11.90 ± 1.65</td>
<td>3.49 ± 1.52</td>
</tr>
<tr>
<td>Multiple</td>
<td>52.31 ± 3.11</td>
<td>59.36 ± 7.72</td>
<td>19.05 ± 2.74</td>
<td>55.85 ± 5.39</td>
<td>15.30 ± 4.01</td>
<td>15.30 ± 4.01</td>
</tr>
<tr>
<td>Ratio s/m</td>
<td>0.13 ± 0.02</td>
<td>0.25 ± 0.07</td>
<td>0.31 ± 0.12</td>
<td>0.21 ± 0.05</td>
<td>0.21 ± 0.05</td>
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</table>

magnitude (high ‘infection pressure’), this was not due to lack of contact between host and parasitoid, but presumably due to a certain resistance of these algae. Finally, all cells were infected. Infection rates of cultures in the exponential growth phase (treatments 3 and 4) were not significantly different from those in the stationary phase (treatments 5 and 6).

Discussion

The experiments with P.diadema showed that the flagellates infect selectively, both interspecifically and intraspecifically. In mixed Coscinodiscus cultures, P.diadema flagellates tended to congregate in the vicinity of C.wailesii rather than C.granii and C.concinnus. This indicates that chemosensory host recognition is enhanced with C.wailesii, which could be caused by higher release rates of organic matter or different exudate composition. The composition of released carbohydrates and free amino acids was found to differ between diatom species (Myklestad, 1974; Martin-Jézéquel et al., 1988), and it appears likely that the organic material released by C.wailesii induced the strongest chemosensory response in P.diadema.

Pirsonia diadema flagellates infect Coscinodiscus spp. cells only through the labiate processes (rimoportulae), structures that form a tubular passage through the siliceous cell wall. On the diatom frustule, labiate processes have been widely recognized as sites where diatoms release organic matter, such as mucus (Crawford, 1973; Andersen et al., 1986; Pickett-Heaps et al., 1986). Coscinodiscus wailesii has up to 400 rimoportulae per valve (Pickett-Heaps et al., 1990), which is at least twice the number in C.granii or C.concinnus cells of comparable size (Hasle and
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Consequently, *C. wailesii* is not only likely to have a higher exudation rate than the other species, but also offers more sites for infections.

Light conditions or temperature did not influence the preference of *P. diadema* for *C. wailesii* compared to *C. granii* (Experiment 1; Figure 1a–d). Infection rates, however, were higher at 15°C than at 10°C. Maximum incidence of infected diatoms in field samples and/or maximum infection rates at temperatures of 15°C or higher have also been reported for several phycomycetes (Gotelli, 1971; Chakravarty, 1974; Raghukumar, 1978; Bruning, 1991c; Wetsteyn and Peperzak, 1991).

No differences in infection rates were found between *C. granii* cultures in the logarithmic and stationary growth phase (Table II), although cells from the stationary phase were more deeply pigmented with denser cell contents, resembling the formation of a resting state (Sicko-Goad, 1986; Sicko-Goad et al., 1986). The number of offspring per infected diatom was higher in the stationary phase (Kühn, 1997b), as the biochemical composition of marine diatoms in the exponential growth phase has been frequently reported to differ markedly from that in the stationary phase (Pugh, 1971; Myklestad, 1974; Taguchi et al., 1987; Martin-Jézéquel et al., 1988). Furthermore, the biochemical and physiological properties of diatoms are known to vary depending on environmental conditions, such as light (Hama et al., 1987; Hama and Handa, 1992) or temperature (Hama et al., 1990; Thompson et al., 1992a,b). Although release rates of organic substances were also found to vary according to growth phase (Eberlein and Brockmann, 1986; Myklestad et al., 1989), any possible changes within *C. granii* apparently did not affect the infection behaviour of *P. diadema*.

It is widely assumed that diatoms grown under suboptimal conditions are more susceptible to infections by parasites or parasitoids. In all experiments, *C. granii* cells maintained in the dark were more rapidly infected by *P. diadema* than those kept under light conditions. The infectivity of several parasitoid protists is known to be affected by darkness: zoospores of parasitoid fungi (chytrids) lose their motility and host recognition ability at very low light levels (Canter and Jaworsky, 1981; Bruning, 1991a), and Schnepf and Drebes (1986) observed that the infection rate of the dinoflagellate *Paulsenella* was higher in the light. In contrast, the infectivity of *P. diadema* flagellates appears to be relatively independent of light conditions. Since *P. diadema* infectivity does not increase in darkness, it can be concluded that higher infection rates in the dark are caused by increased susceptibility of photosynthetically inactive *C. granii* cells.

The susceptibility of *C. granii* to infections by *P. diadema* was significantly higher when experimental cultures had been maintained in darkness 3 days prior to inoculation (Table II). Obviously, even a few hours in the dark affect the relative resistance of *C. granii* to *P. diadema*, as was observed in Experiment 2b, when one light period was replaced by darkness (Table II, Day 1). This contrasts with reports that the photosynthetic capacity of diatoms changes little during the first 3 days in the dark, physiological activity apparently not being reduced (Du Preez and Bate, 1992). Although the physiological activity or photosynthetic potential of *C. granii* during the experiments was not determined, the increased susceptibility within a few hours after transfer into darkness strongly suggests that the physiological
properties of diatoms can change within this short time. Granata (1991) showed that the growth and sinking rates of *Concentricus* responded within 3–4 h to diel periodicity and availability of light, probably caused by short-term changes in physiological properties. Brief exposure of *C.granii* to light at the beginning of a light/dark cycle was probably sufficient for diatoms to recover a certain resistance to infection. In natural populations, *C.granii* should be more susceptible to infections during the night than in the daytime, and also in deeper mixed layers.

It is generally assumed that during active phytoplankton photosynthesis the exudation rates will be higher than in the dark (Mague et al., 1980), so diatoms should be less chemo-attractive in darkness. Malej and Harris (1993), however, determined higher rates of release of carbohydrates by *C.granii* at low light levels than at high irradiance. However, the exact chemical composition of organic material exuded by diatoms and their effects on parasitoid protists remain unknown.

When *P.diadema* flagellates were added to *C.granii*, the majority of single infections on Day 1 (Table II) can be explained by an initial infection by random encounter. *Pirsonia diadema* offspring generally disperse after detaching from the host and do not infect immediately (see Kühn, 1997b). If subsequent infections had spread homogeneously among the diatom population the following day, according to statistical probability, the number of single infections should have again exceeded that of multiple infections (leading to a ratio of single/multiple infections > 1). The predominance of multiple infections, however, strongly suggests that for *P.diadema* flagellates a previously infected *C.granii* and probably leaking cell is more of an attractant than is a non-infected cell, so that after the initial infection by random encounter there is a tendency for recurrent rather than random encounter. Only when diatoms were not exposed to light at any time during the experiment (Table II, treatment 1) did the number of single-infected cells remain higher than that of multiple-infected cells.

But why do some individual *C.granii* cells remain temporarily uninfected? Light microscopically there were no different from other earlier infected cells. Increased susceptibility of non-photosynthesizing diatoms maintained in darkness coinciding with a less discriminant infection behaviour of flagellates suggest that actively photosynthesizing diatoms differ more in their individual physiological properties than non-photosynthesizing cells.

Chisholm et al. (1980) reported a large non-genetic variability in generation times in clonal diatom populations, and concluded 'that population growth rates do not reflect the experience of the individual cells in the population'. Cell cycle phase-specific copepod grazing of phytoplankton was observed by Chang and Dam (1993). The perception of 'individualism' among unialgal cultures is supported by the observation of Du Preetz and Bate (1992) that some individual cells of the diatom *Anaulus australis* were capable of surviving prolonged darkness whereas other cells died. Those approximately 1% of *C.granii* cells that remained uninfected by *P.diadema* until 'infection pressure' finally became too high, are likely to be individually 'at a given moment' physiologically different from other, more susceptible diatoms. The concept of 'individual diatom cells' may explain the temporal resistance (and possibly susceptibility) to infections.
Generally, feeding behaviour studies of heterotrophic organisms, such as dinoflagellates, tend to neglect the physiological condition of the food organism (Jacobson and Anderson, 1986). Discriminant chemoperceptive feeding behaviour in several protozoans was reviewed by Verity (1991). However, if ciliates can respond to the nutritional state of their prey (Verity, 1988), it only seems reasonable to assume that the physiological condition of individual diatom cells will influence the feeding behaviour of selective predators, such as parasitoids. However, it remains difficult to compare the results of the intraspecific selective infection behaviour of *P. diadema* with the feeding or infection behaviour of other heterotrophic protists, especially since in experiments that determined growth rates of parasitoids, multiple infections of hosts were intentionally kept at a minimum as multiple chytrid infections reduced the number of zoospores per sporangium (Bruning, 1991a). In field samples, however, we observed that frequently some cells of the chain-forming marine diatom *Bellerochea malleus* were multiply infected with chytrids, whereas sibling cells were not infected. It was not clear whether infected cells of this species were also more attractive for further infections, or if sibling non-infected cells resulted from divisions of non-infected diatoms. Intraspecific selectivity of parasitoids will increase the chance of non-infected cells remaining uninfected, and will alter the dynamics of infection rates. More studies are needed to find out how common intraspecific selectivity is among parasitoids, both in natural populations and experimental cultures.

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References


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