Infection experiments reveal broad host ranges of *Eurychasma dicksonii* (Oomycota) and *Chytridium polysiphoniae* (Chytridiomycota), two eukaryotic parasites in marine brown algae (Phaeophyceae)

D. G. Müller,^{1*} F. C. Küpper² and H. Küpper¹

¹Faculty of Biology, University of Konstanz, D-78457 Konstanz, Germany and ²Biological Station, CNRS UPR 9042, BP74, F-29682 Roscoff, France.

SUMMARY

Unialgal cultures of the brown alga *Pylaiella littoralis* (L.) Kjellman infected by either *Eurychasma dicksonii* (Wright) Magnus (Oomycota) or *Chytridium polysiphoniae* (Cohn) H. E. Petersen (Chytridiomycota) were used to elaborate the host ranges of these pathogens. Infection experiments with 48 host species covering 13 orders of the Phaeophyceae showed that 45 species were susceptible to attack by *Eurychasma* and 23 to *Chytridium*. The two pathogens showed host-specific differences in generation times: while in *Pylaiella* the shortest cycles were 16 days for *Eurychasma* and 6 days for *Chytridium*, one and five days more, respectively, were required in *Acinetospora*. Heavy parasite attack on the microscopic stages of host species with heteromorphic life histories, like kelps (Laminariales), is documented and discussed as a potential regulatory factor for the population dynamics of macroalgae.

Key words: Chytridiomycota, *Chytridium*, *Eurychasma*, host range, Oomycota, parasitism, Phaeophyceae, *Pylaiella*, temperature tolerance.

INTRODUCTION

Marine brown algae have long been known to be hosts for viruses and various eukaryotic pathogens. While viruses and endophytes are well covered by recent experimental studies (Kawai and Tokuyama 1995; Peters and Ellerts-dóttir 1996; Müller *et al.* 1998), no laboratory studies with fungal and other eukaryotic parasites have been reported.

Pylaiella littoralis (L.) Kjellman has been frequently described with infections by *Eurychasma dicksonii* (Wright) Magnus, which is classified with the Saprolegniales in the Oomycetes (Dangeard 1934; Feldmann 1954; Sparrow 1960; Jenneborg 1977; Wilce *et al.* 1982; Konno and Tanaka 1988; Siemer and Pedersen 1995). Other parasites of *Pylaiella* are the Hyphochytridiomycete *Anisopidium rosenvingii* (H. E. Petersen)

Karling (Petersen 1905; Karling 1943; Sparrow 1960; Küpper and Müller 1999) and the Chytridiomycete *Chytridium polysiphoniae* (Cohn) H. E. Petersen (Petersen 1905; Feldmann 1954; Sparrow 1960).

The spelling of the genus name *Pylaiella* has been the subject of controversy over the years and we have followed here the most recent version recommended by Silva *et al.* (1999).

Numerous reports in the literature, and the compilations by Sparrow (1960) and Jenneborg (1977) document that *C. polysiphoniae* and *E. dicksonii* have a broad host range comprising many brown and even some red algae. As all these reports describe infection structures found in field-collected material, it cannot be decided whether infection symptoms in different host species are caused by one parasite species with a very broad host range, or by a multitude of morphologically similar pathogenic taxa with narrow host ranges. In order to clarify this situation, we established defined cocultures of *P. littoralis* with *C. polysiphoniae* and *E. dicksonii* and used them to examine the parasites' competence to infect clonal cultures of selected host species representing many orders of the brown algae. We also report observations on the temperature tolerance of these pathogens.

MATERIALS AND METHODS

Field specimens of *P. littoralis* infected with *Chytridium* and *Eurychasma* were collected at Aith Voe (Bressay, Shetland) in April 1996 as described by Küpper and Müller (1999). The morphological features of the pathogens (holocarpic character, sporangium morphology and size) corresponded well with the descriptions given by Sparrow (1960) and Karling (1977) for *C. polysiphoniae* and by Jenneborg (1977) for *E. dicksonii*. Small tufts of infected *Pylaiella* were cocultured

*To whom correspondence should be addressed.

Email: <Dieter.Gerhard.Mueller@uni-konstanz.de>

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Table 1. Infection experiments with the parasites *Eurychasma dicksonii* and *Chytridium polysiphoniae* from *Pylaiella littoralis* to other brown algal hosts

Recipient	Life phase, origin	<i>Eurychasma</i>	<i>Chytridium</i>
Ectocarpales			
<i>Acinetospora crinita</i> (Carmichael) Kornmann	New Zealand	+	+
	Australia	+	+
<i>Asteronema rhodoortonoides</i> (Boergesen) Müller et Parodi	Canary Islands	+	– (2)
<i>Botrytella uvaeformis</i> (Pringsheim) Kornmann et Sahling	Helgoland (Germany)	+	+
<i>Botrytella micromora</i> Bory	British Channel	+	– (2)
<i>Ectocarpus siliculosus</i> (Dillwyn) Lyngbye	g, New Zealand	+	+
	sp	+	+
<i>Ectocarpus fasciculatus</i> Harvey	Ireland	+	+
	Brittany	– (3)	+
<i>Feldmannia globifer</i> (Kuetzing) Hamel	Ireland	– (2)	+
<i>Feldmannia irregularis</i> (Kuetzing) Hamel	Chile	+	+
	Canary Islands	+	– (3)
<i>Feldmannia simplex</i> (Crouan) Hamel	Ireland	+	+
<i>Hincksia hinckslae</i> (Harvey) Silva	Brittany	+	+
<i>Hincksia mitchelliae</i> (Harvey) Silva	South Africa	+	+
	Canary Islands	+	+
	Argentina	+	– (2)
	North Carolina	– (2)	– (2)
	Japan	– (2)	– (2)
<i>Hincksia sandriana</i> (Zanardini) Silva	Chile	+	– (2)
<i>Kuckuckia</i> spec.	Norway	+	– (2)
	Yugoslavia	+	– (2)
<i>Kuetzingiella battersii</i> (Bornet) Kornmann	Canary Islands	+	– (2)
<i>Pilayella littoralis</i> (L.) Kjellman	Chile	+	+
	Bering Sea	+	+
<i>Spongonema tomentosum</i> (Hudson) Kuetzing	Canary Islands	+	+
Dictyosiphonales			
<i>Cladothetele decaisne</i> Hooker et Harvey	Patagonia	+	– (3)
<i>Desmotrichum undulatum</i> (J. Agardh) Reinke	Bering Sea	+	– (2)
<i>Dictyosiphon foeniculaceus</i> Greville	Sweden	+	– (2)
<i>Giraudya sphacelarioides</i> Derbes et Solier	Canary Islands	+	– (2)
<i>Litosiphon pusillus</i> (Carmichael ex Hooker) Harvey	Helgoland (Germany)	+	– (2)
<i>Myriotrichia clavaeformis</i> Harvey	Argentina	+	+
Sphacelariales			
<i>Halopteris paniculata</i> (Suhr) Prud'homme van Reine	New Zealand	+	– (2)
<i>Halopteris</i> sp.	Australia	+	+
<i>Sphacelaria rigidula</i> Kuetzing	California	+	– (3)
Tilopteridales			
<i>Haplospora globosa</i> Kjellman	Helgoland (Germany)	– (2)	+
<i>Tilopteris mertensii</i> (Turner in Smith)	Helgoland (Germany)	+	– (2)
Cutleriales			
<i>Cutleria multifida</i> (Smith) Greville	France, Mediterranean Sea	+	– (2)
Syringodermatales			
<i>Syringoderma phinneyi</i> Henry et Müller	British Columbia, Canada	+	– (3)
Chordariales (microthalli)			
<i>Elachista stellaris</i> Areschoug	France, Mediterranean Sea	+	– (2)
<i>Eudesme virescens</i> (Carmichael ex Harvey in Hooker) J. Agardh	Ireland	+	+
<i>Myriogloea chilensis</i> (Montagne) Llaña	Chile	+	+
<i>Stilophora rhizodes</i> J. Agardh	Sweden	+	– (2)
Scytothamnales			
<i>Scytothamnus fasciculatus</i> (Hooker et Harvey) Cotton	Australia	+	– (2)
<i>Splachnidium rugosum</i> (L.) Greville	Tasmania	+	– (3)
Sporochneales			
<i>Carpomitra costata</i> (Stackhouse) Batters	g, France, Mediterranean Sea	+	+
<i>Perithalia caudata</i> (Labillardière) Womersley	g, Tasmania	+	+
<i>Sporochneus pedunculatus</i> (Hudson) C. Agardh	g, France, Mediterranean Sea	+	– (2)

Table 1. Cont.

Recipient	Life phase, origin	<i>Eurychasma</i>	<i>Chytridium</i>
Scytosiphonales			
<i>Endarachne binghamiae</i> J. Agardh	Chile	+	+
<i>Petalonia fascia</i> (O. F. Müller) Kuntze	France, Mediterranean Sea	+	– (2)
<i>Scytosiphon lomentaria</i> (Lyngbye) Link	Japan	+	+
	Australia	+	– (2)
Desmarestiales			
<i>Desmarestia distans</i> (C. Agardh) J. Agardh	Patagonia	+ g, sp.	+ g
<i>Desmarestia munda</i> Setchell et Gardner	g, Vancouver Island, Canada	+	– (2)
<i>Desmarestia viridis</i> (O.F. Müller)	g, Newfoundland	+	+
Laminariales			
<i>Laminaria digitata</i> (Hudson) Lamouroux	Brittany	+ g, sp.	– (2)
<i>Lessonia trabeculata</i> Villouta et Santelices	g, Chile	+ g, sp.	+ on oogonia
<i>Macrocystis pyrifera</i> (L.) C. Agardh	g, Chile	+	– (2)
<i>Saccorhiza dermatodea</i> (De la Pylaie) J. Agardh	g, Newfoundland	+	– (2)
Affiliation unsettled			
<i>Microzonina velutina</i> (Harvey) J. Agardh	New Zealand	– (2)	– (2)

+, infection successful; –, no infection of new host; g, gametophyte; sp, sporophyte (given where known). Figures in parentheses give the number of independently repeated assays with negative result.

with aliquots of a unialgal clonal culture of *P. littoralis* (Pil IR) from Isla Diego Ramirez (Chile, Drake Passage, Müller and Stache 1989). Within several weeks the parasites became established on the new host. In a subsequent step, two Pil IR subclones were initiated which were infected by the parasites in the following way: our *Eurychasma* donor culture started with a Pil IR fragment infected with spores from one *E. dicksonii* sporangium, while our *Chytridium* donor culture originated from a Pil IR fragment exposed to spores from 14 *C. polysiphoniae* sporangia. The two host–parasite cocultures were stable and were used for all the infection experiments. They were maintained in plastic Petri dishes filled with Provasoli enriched seawater (PES) medium (Starr and Zeikus 1993) prepared from artificial seawater (WIMEX, Krefeld, Germany, adjusted to 30% salinity), illuminated with daylight-type fluorescent lamps at an irradiance of $9 \mu\text{E m}^{-2} \text{s}^{-1}$ for 10 h day^{-1} and kept at $10 \pm 1^\circ\text{C}$. Cultures were transferred to fresh medium in 1- or 2-week intervals. Recipient host cultures were unialgal clonal isolates of various brown algae originating from the coasts of the countries listed in Table 1. They were routinely maintained in our laboratory as stock cultures and were kept under the same conditions as the *Pylaiella* parasite donor cultures during this study.

Infection experiments were carried out by coinubation of the *Pylaiella* parasite donor cultures with the prospective host species in 60 mm plastic Petri dishes. Cultures were observed daily under a Leitz (ELVAR) stereo microscope (Wetzlar, Germany) at 63× magnification. A new host was classified as susceptible if sporangia of the pathogen developed on it within 3 weeks. If infection of a new host failed, observation was continued for one more week and the experiment was repeated at least once.

In order to study the temperature dependence of pathogen performance, infection experiments were made at different temperatures with *P. littoralis* and *Acinetospora crinita* (Carmichael) Kornmann as recipients. Plastic Petri dishes containing 10 mL culture medium were placed in different positions along a temperature gradient generated by defined heating and cooling from two ends of an aluminum plate. Experimental temperatures increased in increments of $2.2\text{--}2.5^\circ\text{C}$ and were stable within $\pm 0.5^\circ\text{C}$ of the set value. Infection cycles of the parasites were quantitated as the number of days after which the first empty sporangium was observed on a newly infected algal host.

RESULTS

The cultures confirmed the holocarpic character of both *C. polysiphoniae* and *E. dicksonii*. Infection symptoms remained localized, did not spread to neighboring cells and the parasitic sporangia were formed directly without a mycelium.

The ectoparasite *C. polysiphoniae* occurred on its original host *P. littoralis* (Ectocarpales) at high density, often with two or more sporangia living on one host cell (Fig. 1). Upon maturity, spores escaped through a circular operculum (not shown). In the current infection experiments *Chytridium* attacked the distal uniseriate parts of polystichous thalli, like *Haplospora globosa* Kjellman (Tilopteridales, Fig. 2), and the filamentous microthalli of species with heteromorphic life histories, like the gametophyte of *Carpomitra costata* (Stackhouse) Batters (Sporochneales, Fig. 3). In all cases, *Chytridium* was connected to the host cell by a haustorium. Infected host cells became depleted and eventually died. On all hosts, *Chytridium* sporangia were

spherical and reached different sizes depending on the host (Figs 1–3). Generally, *Chytridium* seemed to prefer actively growing uniseriate filaments and was rarely seen on older or senescent cells or on compact thalli.

The endoparasite *E. dicksonii* showed great affinity for its original host, *Pylaiella* (Fig. 4). The cytoplasm of an infected *Pylaiella* cell was completely consumed by the parasite. Its wall expanded and eventually formed an *Eurychasma* sporangium with one or more beak-like extensions which differentiated into apertures for spore release. Host thalli easily fragmented by breakage at the sites of empty parasite sporangia. In *Pylaiella* and many other hosts, empty *Eurychasma* sporangia showed the 'net' structure (Figs 4, 6, 9, 10, 12, 13), a characteristic ornamentation of the sporangium wall mentioned by earlier workers (Sparrow 1960, p. 818; Jenneborg 1977).

Eurychasma attacked filament cells of the Ectocarpales, the filamentous microthalli of heteromorphic hosts (Figs 5–9) and juvenile sporophytes of *Desmarestia* (Fig. 8). Furthermore, it infected individual cells within compact macrothallus tissue in members of the orders Dictyosiphonales (Fig. 10) and Laminariales (Figs 11, 13, 14) and their microscopic gametophyte stages (Figs 12, 15). *Eurychasma* also invaded the prominent apical cells as well as subapical and differentiated tissue cells in members of the order Sphacelariales (Figs 16–18).

Table 1 summarizes the results of the infection experiments. A total of 48 host species from 13 orders of the Phaeophyceae were examined. Of these, 45 species were susceptible to infection by *Eurychasma* and 23 to *Chytridium*. Only *Microzonia velutina* (Harvey) J. Agardh was resistant to both parasites. In *Ectocarpus fasciculatus* Harvey, *Hinckesia mitchelliae* (Harvey) Silva, *Feldmannia irregularis* (Kuetzing) Hamel and *Scytosiphon lomentaria* (Lyngbye) Link isolates of different geographic origin showed differential susceptibility to the two pathogens.

Figure 19 shows the effect of temperature on the infection of *P. littoralis* and *Acinetospora crinita* by *Eurychasma* and *Chytridium*. Judged by visual inspection of inoculum development, both algae showed maximum growth around 15°C and died at 23°C. *Eurychasma* infected both host species from +4°C to their lethal limit at 23°C. The most rapid development of the parasite occurred at 12°C. At this temperature the complete developmental cycle of *Eurychasma* required 14–15 days in *Pylaiella* and 1–2 days more in *Acinetospora*. *Chytridium* had a narrower temperature range, it persisted on both hosts between 4 and 15°C. At 15°C, with maximum growth rates of the algal hosts, the virulence of *Chytridium* appeared much reduced and only a few fungal sporangia were seen; at 16°C the parasite died. Development of *Chytridium* depended strongly on the host. It was most rapid in *Pylaiella* at 11°C, where a full cycle took 6 days, while the shortest cycles in *Acinetospora* required 11 days at 10°C.

DISCUSSION

The present study represents the first experimental investigation of eukaryotic parasites in brown algae under laboratory conditions. Both *Chytridium* and *Eurychasma* can infect many hosts within a broad taxonomic range and from many coasts of the world. This characterizes them as ubiquitous and cosmopolitan marine pathogens.

In our experiments the severity of infection symptoms differed between various hosts and, occasionally, also in repetitions of the same combination. This may reflect interference of the epibiotic bacterial flora with the settlement of parasite spores and other unknown factors that may modify the virulence of the parasites and the susceptibility of the hosts. Therefore, the negative results in the present study (i.e. resistance of certain hosts to infection) must be considered with caution as

Figs 1–18. Disease symptoms caused by *Chytridium polysiphoniae* and *Eurychasma dicksonii* in laboratory cultures of various brown algal hosts. 1–3. *Chytridium* infection on various hosts. 1. Parasite sporangia on *Pylaiella littoralis*. Host cells are attacked by two or more parasites (arrowheads). 2. *Chytridium* on a uniseriate apical part of the *Haplospora globosa* thallus. 3. *Chytridium* on a gametophyte of *Carpomitra costata*. 4–18. *Eurychasma* infection of various hosts. 4. *Pylaiella littoralis*. Host cells with young parasite syncytia (arrowheads) and empty sporangia of the parasite. 5. Microthallus of *Scytosiphon lomentaria*. 6. Microthallus of *Petalonia fascia*. 7. Heavy infestation of older gametophyte culture of *Desmarestia distans* with young sporophytes (arrowheads indicate empty parasite sporangia). 8. A parasite syncytium in a cell of a *D. distans* sporophyte. 9. Parasite sporangium on a gametophyte of *D. distans*. 10. An empty parasite sporangium on *Desmotrichum undulatum*. 11. Host cells of *Lessonia trabeculata* with young parasite syncytia and empty sporangium in juvenile host sporophyte. 12. Parasite sporangia on host gametophyte of *L. trabeculata*. 13. Cells of juvenile sporophyte of *Laminaria digitata* with various stages of parasite development, including fragmentation of the host (14). 15. Parasite in gametophyte cells of *Saccorhiza dermatodea*. 16. The parasite is killing apical and subapical cells of *Stypocaulon scoparia* (arrowheads). Damage by loss of apical cell (asterisk) repaired by regeneration. Immature and empty parasite sporangium emerging from a thallus cell of *S. scoparia* (16, center and 17). 18. An early parasite stage developing in an apical cell of *Halopteris paniculata*. Figs 1, 4 from living material, all others from permanent voucher slides of the current infection experiments. Bar = 100 µm applies to Figs 1–3, 4–18, respectively.

Fig. 19. Correlation between temperature and development of (a) *Chytridium* and (b) *Eurychasma* on the hosts *Pylaiella littoralis* (□) and *Acinetospora crinita* (●). Infection cycle: minimum number of days required for the maturation and discharge of newly formed parasite sporangia. Data were processed by the program Microcal Origin 4.1, and graphs were generated by square regression. Lethal limits for *Chytridium* (----) and algal hosts (- · - · -).

the true host ranges of both pathogens may be even broader. Nevertheless, it seems obvious that *Eurychasma* has a significantly broader host range than *Chytridium*.

None of the host cultures in the present infection experiments was killed by *Eurychasma* or *Chytridium* and all host-pathogen combinations arrived at a state of coexistence. We noticed two obvious mechanisms that allowed the hosts to escape from parasite attack. The fragmentation of host thalli caused by *Eurychasma* produced vegetative propagules, which gave the host the chance to drift to new habitats with no or reduced parasite pressure. Mass developments of drifting *Pylaiella* have been attributed to this effect (Wilce *et al.* 1982). The upper lethal limit of 16°C for *Chytridium* allows many hosts to survive and grow without the parasite at higher temperatures. This effect is likely to create seasonal fluctuation of the host-pathogen interaction in natural habitats. We did not observe resting stages or sexuality in the current parasite cultures.

Several brown algal orders exhibit heteromorphic life histories: Chordariales, Dictyosiphonales, Scytosiphonales, Scytotamniales, Desmarestiales, Sporochinales and Laminariales. In these groups either the gametophytes or sporophytes are microscopic in size, but constitute obligatory parts of the life cycles. It is

difficult or impossible to detect, identify and study such microscopic stages in their natural habitats and, consequently, reports of infections on such stages are completely lacking in the phycological literature. Our study has now revealed that parasitic pressure on the microscopic stages of heteromorphic life histories is likely to occur. It is presently impossible to fully comprehend the consequences of this finding. Depending on circumstances, parasite attack on these cryptic stages may reduce or stimulate the recruitment of macrothalli.

For methodical reasons, the present study could not cover the entire taxonomic range of the Phaeophyceae and members of the Fucales and Durvillaeales with economically and ecologically important representatives were not included. Other groups, like Notheiales or Ascoseirales, were not available for the experiments. Further studies are needed to determine if, and to what degree, these groups are also subject to parasite attack.

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