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Cross-infection experiments with Australian *Perkinsus* species

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ABSTRACT: Infections of *Perkinsus* spp. were initiated in 9 mollusc species using zoospores from cultured prezoosporangia held in petri dishes containing seawater We transferred parasites isolated from bivalves to gastropods and vice versa. Isolates from tropical, subtropical and temperate hosts were transferred to mollusc hosts from different climate origins. Some hosts, e.g. *Saccostrea commercialis*, were largely refractory, with only light infections developing in a few molluscs. Other hosts, e.g. *Anadara trapezia* and *Pinctada sugillata*, became heavily infected. An experimental infection in one *A. trapezia* endured in the laboratory for 7 mo.

INTRODUCTION

Perkinsus marinus (Mackin et al. 1950) was described from *Crassostrea virginica*. Ray (1954) reported infections in *Ostrea lurida* and White et al. (1987) recently found it in a pyramidellid gastropod that had fed on an infected *C. virginica*. The only other named species of *Perkinsus*, *P. olseni*, was described from the gastropod *Haliotis rubra* by Lester & Davis (1981). Prezoosporangia, belonging to what appear to be other *Perkinsus* species, have been observed in 21 species of bivalve in North America (Andrews 1954, Ray 1954), 6 in the Mediterranean (da Ros & Canzonier 1985) and 30 in Australia (Goggin & Lester 1987).

Structural differences that would enable the separation of *Perkinsus* species have not yet been recognised (Perkins in press) and cross-infection experiments with various isolates have, to date, been unsuccessful, suggesting strong host specificity. Experimental transmission between specimens of *Crassostrea virginica* has been accomplished by several methods: by adding minced, infected, oyster tissue to the aquarium (Ray & Mackin 1954); by putting infected oysters in the aquarium alongside uninfected individuals (Ray 1954); by adding water from infected aquaria (Mackin 1952); and by injecting infected tissue (Mackin et al. 1953). However, Ray (1954) was unable to transfer the parasite via injection from *Macoma balthica* to either *Crassostrea virginica*, *Venus mercenaria* or *Mya arenaria*, or from *C. virginica* to *V. mercenaria* or *M. arenaria*. He concluded that this was due to the rigid host specificity of the parasite. Andrews & Hewatt (1957) also injected infected *C. virginia* tissue into *V. mercenaria* and furthermore fed them tissue from infected *M. balthica*, but were unable to detect any infection after 1 mo. Lester & Davis (1981) were unable to transmit *P. olseni* from *Haliotis rubra* to the Australian commercial oyster *Saccostrea commercialis*.

The work described below was undertaken to clarify the species affinities of the isolates of *Perkinsus* spp. we found in Australia (Goggin & Lester 1987, Lester et al. 1988).

MATERIALS AND METHODS

Isolates of *Perkinsus* spp. were obtained from the following molluscs and locations: *Tridacna gigas*, Orpheus Island, Queensland (Qld); *Chama pacificus* and *Tridacna maxima*, Heron Island reef, Qld; *Barbatia corallicola* and *T. crocea*, Lizard Island reef, Qld; blood cockle *Anadara trapezia*, Wynnum in Moreton Bay, Qld (all bivalves); and abalone *Haliotis* spp. (gastropods), western Gulf of St Vincent, South Australia. Uninfected molluscs were obtained from Lizard Island, (*Saccostrea cuccullata*), Moreton Bay (*Pinctada sugillata*, hairy mussel *Trichomya hirsuta* [bivalves], *Pyrazus ebinenus* [gastropod]), Deception Bay in Moreton Bay (*Anadara trapezia*), Terranora Lakes, New South Wales (N.S.W.) (*Isognomon* sp., *Saccostrea commercialis* [bivalves]) and West Island, South Australia (*Haliotis* spp.) (Fig. 1, Sites 1 to 7).

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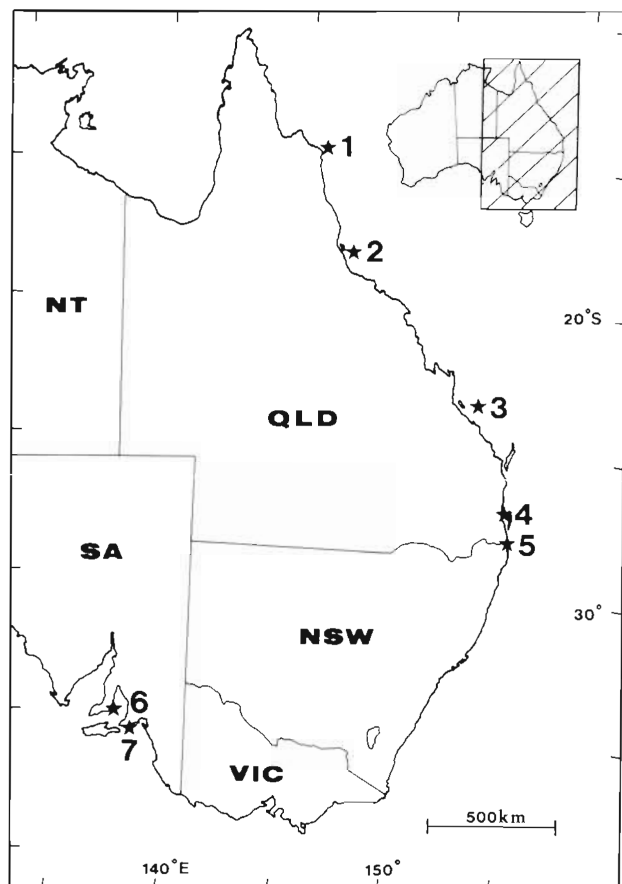

Fig. 1. Map of eastern Australia showing the 7 sites where experimental molluscs were collected. 1: Lizard Island; 2: Orpheus Island; 3: Heron Island; 4: Moreton Bay; 5. Terranora Lakes; 6: western Gulf of St Vincent; 7: West Island

In preliminary experiments, tissue from *Chama pacificus* infected with *Perkinsus* sp. was injected into *Saccostrea commercialis* through a 26G needle. Two tests were done at 20 °C and two at 27 °C (Table 1).

All further infections with *Perkinsus* spp. were initiated as follows: parasites were isolated from infected molluscs by culturing tissue in fluid thioglycollate medium (Ray 1966). After 4, 5 or 7 d, infected tissue was removed from the medium and transferred to 10 cm diam. petri dishes of seawater. Heavily infected tissue was teased apart to liberate prezoosporangia. Excess tissue was removed and prezoosporangia allowed to settle on the bottom of the dish for up to 12 h. The surface water was then decanted to leave the prezoosporangia, which adhered to the glass. Seawater was changed daily by upending the dish and refilling. The development of prezoosporangia to zoosporangia was monitored with a compound microscope. When motile zoospores were visible within zoosporangia the seawater was poured off and dishes immersed in aquaria holding test molluscs. At the same time, flow-through water in the aquaria was stopped. Dishes were left until all zoosporangia had discharged (up to 96 h) to allow for maximum infestation. Water in the control aquarium of each experiment was also turned off during the infection period. Test, and an equal number of control, molluscs were then maintained at either 20 °C (all experiments with abalone and some with *Anadara trapezia*, see Table 1) or 27 °C (all other experiments). Molluscs were killed after 2 to 196 d. For individual molluscs, infections were assessed essentially as described by Quick (1971). We defined 7 levels of infection = Level 0: 0 hypnospores per sample; Level 1: 1 to 10 per sample; Level 2: 11 to 100 per sample; Level 3: 101 to 1000 per sample; Level 4: 31 to 300 per field 5 mm in diam.; Level 5: 301 to 1000 per 5 mm field; and Level 6: more than 1001 per 5 mm field (ca 0, 0.006, 0.04, 0.8, 8, 33 and 102 hypnospores mm^{-2}, respectively). Samples included the whole mollusc, except for abalone where a standard tissue section was taken just posterior to the head on the left side. This section included pieces of gill, foot, digestive gland, muscle and mantle. For each experiment, an average level of infection (between 0 and 6) was calculated as the sum of infection levels of all individuals divided by the number of molluscs exposed to infection (Table 1). After thioglycollate culture, all isolates were allowed to develop in seawater as a test of their viability.

RESULTS

The introduction of petri dishes coated with *Perkinsus* spp. into aquaria was an effective and rapid method of producing infections in receptive molluscs without compromising their fitness. Most inter-specific infections attempted using this method were successful (Table 1) although the level of infection and number of positive molluscs varied. *Saccostrea commercialis* and *Trichomya hirsuta* were largely refractory. Few of these became infected and most that did had low levels of infection (Levels 0 to 3). The heaviest infections seen in *S. commercialis* were in individuals held for longer than 2 mo and infected with *Perkinsus* sp. from *Anadara trapezia* (see Table 1) where 1/10 and 3/20 oysters, respectively, registered Level 4 infections. *A. trapezia* and *Pinctada sugillata* were particularly susceptible to infection. *Perkinsus* sp. originally isolated from the bivalves *A. trapezia, Chama pacificus, Tridacna gigas, T. crocea* and *T. maxima* were able to be transferred to, and developed in, the gastropods *Haliotis laevigata, H. scalaris* and *Pyrazus ebinenus* (Table 1). Infections isolated from abalone held at 20 °C and cultured at 27 °C infected Moreton Bay bivalves. *Perkinsus* spp. isolates taken from *Chama pacificus* from Heron Island were capable of infecting a range of Moreton Bay bivalves.

Table 1. *Perkinsus* spp. Infection attempts with isolates. Table lists: recipient species; no. recipient hosts infected/no. exposed; time after infection that molluscs were killed to check infection level (in parentheses if all negative); average infection level (see text for derivation) and size range. Arrows indicate passage of parasite isolates; RKA: remainder killed at day no. indicated

Recipient species	No. infected/ no. exposed	Infection duration (d)	Average infection level	Size range (mm)
Source species: *Tridacna gigas*				
Pinctada sugillata	15/15	5, 10, 15	4.8	33–60
→ *Saccostrea commercialis*	16/16	28, 42, 84	2.9	20–37
→ *Pyrazus ebinenus*	12/13	28, 42	2.7	ca 50
→ *P. sugillata*	15/15	5, 10, 15	4.0	34–69
→ *Anadara trapezia*	15/15	5, 10, 15	4.7	31–40
Source species: *Tridacna maxima*				
Saccostrea cucculata	0/10	(5, 10, 15)	0	14–33
Source species: *Tridacna crocea*				
Saccostrea cucculata	0/10	(5, 10, 15)	0	10–30
Source species: *Anadara trapezia*				
Pinctada sugillata	15/15	5, 10, 15	2.9	33–62
→ *P. sugillata*	14/15	5, 10, 15	2.8	21–71
Anadara trapezia ←	15/15	5, 10, 15	3.9	33–44
→ *A. trapezia*	32/32	up to 105[a]	5.3	25–45
A. trapezia ←	28/28	up to 60[a]	5.6	29–44
A. trapezia ←	29/29	21, 56, 84	2.4	32–49
A. trapezia ←	32/32	21, 56, 84	3.8	35–50
→ *A. trapezia*	20/20	RKA 53[a]	5.2	19–41
→ *A. trapezia* (20 °C)	32/32	RKA 196[a]	5.3	25–47
→ *A. trapezia*	35/35	28, 56, 84	4.3	33–49
→ *A. trapezia*	31/31	28, 56, 84	5.2	33–51
A. trapezia ←	20/20	RKA 25[a]	5.4	34–45
A. trapezia ←	16/16	RKA 23[a]	5.5	16–48
Haliotis scalaris	3/3[c]	32	3.7	43–72
→ *P. sugillata*	7/7	16–22	2.7	45–54
→ *Saccostrea commercialis*	13/16	16–22	0.9	14–49
S. commercialis	10/10	62	1.9	20–60
S. commercialis	20/20	79	2.0	20–52
Source species: *Chama pacificus*				
Saccostrea commercialis[b] (20 °C)	1/12	(5), 10	0.1	17–28
S. commercialis[b]	1/12	(5), 10	0.1	14–35
S. commercialis[b] (20 °C)	0/13	(RKA 23[a])	0	18–30
S. commercialis[b]	0/12	(up to 18[a])	0	16–25
S. commercialis	3/16	21, 35	0.3	16–30
S. commercialis	3/36	(3), 5, 7, 10, 15, 20, 30	0.1	20–43
Trichomya hirsuta	5/10	5, (10), 15	0.5	12–20
Pinctada sugillata	6/6	5, 10, 15	2.7	48–65
S. commercialis	10/10	5, 10, 15	1.8	21–42
Isognomon sp.	4/4	5, 10, 15	2.5	96–126
P. sugillata	12/15	1, 2, 3, 5	1.3	31–52
→ *T hirsuta*	1/15	(5), 10, (15)	0.1	11–26
Source species: *Barbatia corallicola*				
Saccostrea cucculata	2/10	5, 10, (15)	0.2	14–26
Source species: *Haliotis laevigata*				
Pinctada sugillata	13/15	5, 10, 15	1.7	32–73
→ *Haliotis laevigata*	1/1[c]	28	4.0	102
→ *H. cyclobates*	1/1[c]	28	3.0	68
H. laevigata	1/2[c]	(5), 40	0.5	63
H. laevigata	4/4[c]	27	3.0	44–108
H. scalaris	3/3[c]	27	3.0	43–72
P. sugillata	17/19	15	1.8	30–58
Anadara trapezia	7/7	20–97	3.9	34–47
Source species: *Haliotis cyclobates*				
Pinctada sugillata	9/9	15	1.7	30–64

[a] As mortalities occurred
[b] Injection experiment
[c] No controls: surveys by Lester & South, Australian Department of Fisheries (unpubl.), found no infection at West Island, the origin of the abalone

Perkinsus sp. isolated from *T. gigas* from Orpheus Island was also able to infect Moreton Bay bivalves held at 27 °C No infections were found in any of the controls.

Very low levels of infection were transferred in 2 preliminary trials by injecting infected, minced tissue of *Chama pacificus* into *Saccostrea commercialis,* though injection of tissue often led to death of the host. Two experiments conducted at Lizard Island Research Station were unsuccessful; infection was not transferred to *S. cucullata* using zoospores cultured from *Tridacna crocea* or *T. maxima* (Table 1).

Infections were detected in experimental molluscs *Pinctada sugillata* after 1 d (2/4 had Level 1 infections, Table 1). One *Anadara trapezia,* experimentally infected with *Perkinsus* sp. from the same host species, remained infected for 7 mo in the laboratory at 20 °C.

In several cases, experimentally infected molluscs were used as a source of *Perkinsus* sp. to successfully infect further molluscs. For example, an isolate from *Tridacna gigas* was grown in *Pinctada sugillata,* reisolated and then used to infect other *P. sugillata* and 3 other species of mollusc (Table 1). An isolate, originally from *Anadara trapezia* (from Wynnum), was passaged 7 times through previously uninfected bivalves (Table 1).

Perkinsus schizonts in experimentally infected *Anadara trapezia* were almost always subepithelial. Few were observed deep in the tissue of the host.

DISCUSSION

The advantages of our infection method are that large numbers of animals can easily be infected with no mechanical damage to the host, and that high levels of infection can be achieved. The poor success rate of our initial inoculation experiments is in agreement with that of Ray (1954). At the time of his experiments he was unaware that the parasite produced zoospores and so, was unable to test this method of infection. Zoospores were first found by Perkins & Menzel (1966).

The lack of schizonts deep in the tissue of *Anadara trapezia* suggests that infection in this host is via penetration and not per os (Lester et al. 1989). *Perkinsus marinus,* however, has been found in experimentally infected oysters *Crassostrea virginica* in the digestive epithelium, from the stomach to the rectal area and is assumed to enter the host primarily with the feeding current (Mackin 1951). It is not clear why schizonts in *A. trapezia* were only found just beneath the epithelium in our experimental infections, whereas in *C. virginica* and in naturally infected hosts we have examined, including *A. trapezia,* they multiply throughout the host tissues.

The origin of the parasite (i.e. temperate, tropical or subtropical) did not affect the ability of the isolate to be transferred to molluscs from different localities. Isolates from gastropods *(Haliotis laevigata)* were transferred to bivalves, and vice versa, indicating a low level of host specificity for this (these) parasite(s). We found that the histological appearance of natural infections varied greatly in different host species and suggested at least 2 *Perkinsus* species were present on the Great Barrier Reef. We now plan to evaluate the histology of experimental infections and to compare the rates of development of prezoosporangia in seawater in order to develop criteria to separate species and strains. Serological and genetic studies will be possible once we have sufficient material in axenic culture.

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