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The origin of two crayfish plague (*Aphanomyces astaci*) epizootics in Finland on noble crayfish, *Astacus astacus*

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A fungus identified as *Aphanomyces astaci* was isolated from two crayfish plague epizootics among noble crayfish, *Astacus astacus,* in Central Finland. The isolated fungal strains from Vaikkojoki, Kaavi and Iso-Suojärvi, Saarijärvi were able to kill healthy *A. astacus* crayfish in aquarium experiments and the fungus could be reisolated from the diseased animals. The genomic DNA of the isolated strains were characterized by applying a polymerase chain amplification technique with arbitrarily primed DNA. One strain was identical, i.e. showed an identical DNA band pattern, with an *A. astaci* strain earlier isolated from signal crayfish, *Pacifastacus leniusculus*. The latter strain has been the cause of several crayfish plague outbreaks in Sweden. These data suggest that the introduction of North American crayfish into Finland has resulted in the transmission of the disease to populations of the native crayfish, *A. astacus*.

1. Introduction

Crayfish plague is the most serious disease affecting freshwater crayfish and is the cause of the eradication of a large number of European crayfish populations and of large economic losses (Alderman & Polglase 1986, Cerenius & Söderhäll 1992, Söderhäll & Cerenius 1992). Consequently, different control measures have been taken in several European countries to prevent the transmission of the organism causing these losses, the crayfish plague fungus, *Aphanomyces astaci* (Schikora). Different North American crayfish species, e.g. *Pacifastacus leniusculus* (Dana), were introduced since these are more resistant to this pathogen than the European species. However, North American crayfish carry the fungus and they transfer the disease to susceptible indigenous crayfish species such as noble crayfish, *Astacus astacus* (Söderhäll & Cerenius 1992). In addition, it is well established that North American crayfish themselves easily succumb to the crayfish plague infection if the animals are stressed (Söderhäll & Cerenius 1992).

The crayfish plague fungus was probably introduced to Europe from North America around 1860 (Alderman & Polglase 1986) although the fungus was not isolated and identified at that time. It is likely that this pathogen was re-introduced into Europe at several occasions with stockings of American crayfish. So far, three major groups or clones of the fungus have been identified with the aid of DNA techniques (Huang *et al*. 1994, Dieguez-Uribeondo *et al.* 1995, Lilley *et al.* 1997a). One group was isolated from a number of crayfish plague outbreaks on *A. astacus* in Sweden. The oldest of these Swedish isolates is from 1962 and this clone has also been recovered from another native Eurasian species, namely *A. leptodactylus* in Turkey. Perhaps this group represents an early introduction of the fungus to Europe (Huang *et al.* 1994). A second group of the fungus was isolated from a *P. leniusculus* population which originated from lake Tahoe, USA, and was widely used to stock European waters. From 1970 onwards, fungal isolates which belong to the second group ("the signal crayfish group") were isolated from native crayfish in Sweden, which suggests that the large-scale stockings of signal crayfish which occurred in Sweden from 1969 and onwards resulted in the spread of the second clonal type (Huang *et al.* 1994). A third clone of *A. astaci* was recently identified in another introduced American crayfish species, *Procambarus clarkii* (Dieguez-Uribeondo *et al*. 1995).

This study was undertaken as an effort to isolate the source of crayfish mortalities in two Finnish lakes and since the organism responsible for the mortalities was identified as *A. astaci,* DNA fingerprinting was carried out to trace the origin of the disease.

2. Materials and methods

2.1. Isolation of *A. astaci* from diseased crayfish

The fungus was isolated from *A. astacus* crayfish essentially as described in Cerenius *et al*. (1988). An isolate from the river Vaikkojoki, Kaavi, Kuopio province assigned with the number K121, and an isolate from the lake Iso-Suojärvi, Saarijärvi, province of Central Finland (K136) were chosen for a more detailed study.

2.2. DNA preparation and PCR

The mycelia were cultured in liquid PG-1 medium (Cerenius *et al.* 1987) and DNA extracted from about 0.5g of mycelium using a Nucleon II kit (Scotlab, Strathclyde, UK) according to the manufacturers instructions for fungal DNA isolation. The yield was typically 100 µg of DNA.

The PCR reactions were carried out as described in Huang *et al.* (1994) except that the primer concentration was 200 nM. The enzyme was purchased from U. S. Biochemical Corp. and the primers for random amplification of polymorphic DNA (RAPD) were identical to the primers used in Huang *et al.* (1994).

2.3. Infection experiments

Healthy *A. astacus* crayfish (Kulebrokräftan, Kalmar, Sweden) were incubated with K121 or K136 zoospores as described in similar experiments using other *A. astaci* strains in Rantamäki *et al.* (1992). For each fungal strain 8 animals in two separate aquaria were infected. The Swedish *A. astaci* strain Hö was used in control experiments run in parallel.

3. Results

From crayfish collected during outbreaks of crayfish plague in two different water systems in Central Finland, a fungus was consistently isolated from diseased animals. The morphology of hyphae, sporangia, primary spores and zoospores was consistent with the characters described as diagnostic for the genus *Aphanomyces* (Scott 1961). No sexual structures were observed. Two of the isolates, K121 and K136, were chosen for further studies. Infection experiments using disease-free noble crayfish held in aquaria were performed in order to confirm the pathogenicity of the isolates. Both isolates caused a 100% mortality in the aquarium experiments and the fungus was recovered from the abdominal cuticle of these crayfish. Thus, based on the morphology and the pathogenicity towards noble crayfish, the two new isolates were assigned to belong to the species *A. astaci*.

The crayfish plague fungus is clonally propagated and the RAPD-PCR technique can be used to discriminate between different clones of the *A. astaci* (Huang *et al.* 1994, Dieguez-Uribeondo *et al*. 1995) and other *Aphanomyces* species parasitic on e.g. fish (Lilley *et al.* 1997b). RAPD-PCR

analysis showed that the Finnish isolates belong to two different clones (Fig. 1). The K121 isolate is identical to the previously characterized Pl strain and related isolates which were isolated from either signal crayfish brought to Sweden to stock natural waters or from noble crayfish after large-scale stockings of signal crayfish had taken place in Sweden. K136 belongs to a different group of isolates and represents a clone which was present in Scandinavia in 1962 or earlier (Huang *et al.* 1994). Thus, the plague epizootics in these two water systems have different origins.

4. Conclusion

Signal crayfish, a species of North American origin, is used to stock many natural waters in Finland and Sweden. The presence of the "signal crayfish-clone" of the crayfish plague fungus on diseased noble crayfish, as was the case in Vaikkojoki, demonstrates that the introduction of signal crayfish did indeed contribute to the spread of the fungus. Whether the epizootic in Vaikkojoki was caused by a direct transfer of the fungus from signal crayfish present in the river or indirectly by noble crayfish by a series of transmissions of the disease between noble crayfish populations after the original transfer of the parasite from signal crayfish to the native species can not be established without further data. In any case, it is obvious that a new clone of the parasite has been introduced due to stockings of *P. leniusculus* in Finland. We have recently shown that the crayfish plague outbreaks in England most likely are due to the introduction of *P. leniusculus*, since 2 strains of the crayfish plague fungus isolated from native crayfish were shown to originate from introduced signal crayfish (Lilley *et al.* 1997a). This introduction caused the eradication of many populations of native crayfish, an outcome which suggests that great care should be executed in allowing any movement of alien crayfish.

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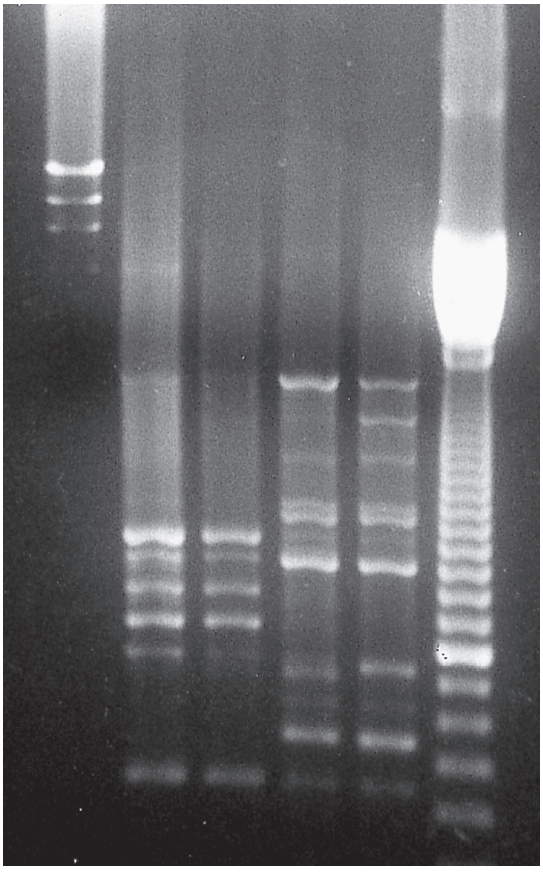

Fig. 1. RAPD-PCR profiles obtained with primer B01. From the left lambda *Hin*dIII marker, *A. astaci* isolates K121, Pl, K136 and L1 and to the right a 100bp-ladder (Pharmacia). Note the similarity between strains K121 and Pl and the similarity between K136 and L1.

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