Journal of Invertebrate Pathology 145 (2017) 68–71

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

Apparent interspecific transmission of *Aphanomyces astaci* from invasive signal to virile crayfish in a sympatric wild population

J. James [a,*], A. Mrugała [b], B. Oidtmann [c], A. Petrusek [b,1], J. Cable [a,1]

[a] School of Biosciences, Cardiff University, Cardiff CF10 3AX, United Kingdom
[b] Department of Ecology, Faculty of Science, Charles University, Prague, Viničná 7, CZ-12844 Prague 2, Czech Republic
[c] Centre for Environment, Fisheries and Aquaculture Science (Cefas), The Nothe, Weymouth, Dorset DT4 8UB, United Kingdom

ARTICLE INFO

Article history:
Received 26 May 2016
Revised 14 February 2017
Accepted 15 February 2017
Available online 17 February 2017

Keywords:
Pacifastacus leniusculus
Orconectes cf. *virilis*
Wildlife disease
Transmission pathways

ABSTRACT

The crayfish plague pathogen (*Aphanomyces astaci*) causes mass mortalities of European crayfish when transmitted from its original North American crayfish hosts. Little is known, however, about interspecific transmission of the pathogen between different American crayfish species, although evidence from trade of ornamental crayfish suggests this may happen in captivity. We screened signal and virile crayfish for *A. astaci* at allopatric and sympatric sites in a UK river. Whilst the pathogen was detected in signal crayfish from both sites, infected virile crayfish were only found in sympatry. Genotyping of *A. astaci* from virile crayfish suggested the presence of a strain related to one infecting British signal crayfish. We conclude that virile crayfish likely contracted *A. astaci* interspecifically from infected signal crayfish. Interspecific transmission of *A. astaci* strains differing in virulence between American carrier species may influence the spread of this pathogen in open waters with potential exacerbated effects on native European crayfish.

1. Introduction

The crayfish plague agent, *Aphanomyces astaci,* is arguably one of the most devastating invasive parasites in European freshwaters (Lowe et al., 2004; DAISIE, 2009). Since its first introduction in the mid-19th century (Alderman, 1996; Holdich, 2003), the pathogen has spread throughout Europe, facilitated in recent decades by movements of invasive North American (henceforth referred to as American) crayfish (Souty-Grosset et al., 2006; Holdich et al., 2014; James et al., 2014). Whilst American crayfish are often asymptomatic carriers of *A. astaci* infection, the disease is usually lethal in European species (Unestam and Weiss, 1970; Diéguez-Uribeondo et al., 1997; Bohman et al., 2006; Kozubíková et al., 2008). Once introduced, *A. astaci* can spread rapidly, transmitted through zoospores that are released into water (Oidtmann et al., 2002) and can survive for at least 14 days (CEFAS, 2000). Spores are mainly released during host moulting or death (Svoboda et al., 2013), and within a cadaver *A. astaci* can remain viable for several days (Oidtmann et al., 2002). Therefore, the movement of infected carcasses by predators could facilitate pathogen dispersal. If fish ingest infected tissue, the pathogen can even survive passage through the gastro-intestinal tract, providing an additional transmission pathway (Oidtmann et al., 2002).

Whilst the transmission of *A. astaci* from non-native American to European crayfish has been widely documented (e.g. Alderman et al., 1990; Diéguez-Uribeondo et al., 1997; Vennerström et al., 1998; Bohman et al., 2006), little is known about interspecific pathogen transmission between these invasive carriers. Until now, four different *A. astaci* genotype groups have been isolated in Europe; group A was obtained from infected native European crayfish (*Astacus astacus* and *A. leptodactylus*) and groups B, D and E from different American crayfish species (*Pacifastacus leniusculus*, *Procambarus clarkii* and *Orconectes limosus*, respectively) (Svoboda et al., 2017). The genotype groups infecting additional *A. astaci* carriers known from European waters, calico (*Orconectes immunis*), marbled (*Procambarus fallax* f. *virginalis*) and virile (*Orconectes* cf. *virilis*) crayfish (Filipová et al., 2010; Schrimpf et al., 2013; Keller et al., 2014; Tilmans et al., 2014), are so far unknown. Existing data suggest that *A. astaci* genotype groups are host-specific among American crayfish (Grandjean et al., 2014). There is no evidence of strains transmitting between these crayfish in the wild, although it seems to occur in the aquarium trade (Mrugała et al., 2015).

Here, we investigate interspecific transmission of *A. astaci* upon contact of two potential carrier species. Signal crayfish are widespread across the UK (James et al., 2014) and were initially stocked

* Corresponding author.
E-mail address: jamesj12@cardiff.ac.uk (J. James).
[1] Joint last authors.

http://dx.doi.org/10.1016/j.jip.2017.02.003
0022-2011/

into the River Lea during the mid-1970s (Almeida et al., 2014). Conversely, virile crayfish are only found in the Lea catchment in the UK (James et al., 2014), possibly unintentionally introduced there around 2004 (Ahern et al., 2008). The two species have been co-existing since at least 2011 (James et al., 2016). Virile crayfish in this river, as well as in a population from the Netherlands, have been reported to carry *A. astaci* (Tilmans et al., 2014), and it was suggested that these crayfish were already infected prior to introduction. Here, we tested the alternative hypothesis that virile crayfish contracted *A. astaci* from co-existing signal crayfish, by evaluating the distribution of the pathogen at allopatric and sympatric sites within the River Lea and an adjacent lake in London, and by genotyping the pathogen from infected host specimens.

2. Methods

Invasive signal crayfish and virile crayfish were collected from the River Lea and an adjacent lake in London, UK, during September 2014. Using baited traps employed over two consecutive nights and checked daily, animals were caught from allopatric (Lat/Long: 51°45′14″N/000°00′16″E, 51°42′29″N/000°01′16″W for signal and virile crayfish respectively, n = 30 for each species) and a sympatric site (Lat/Long: 51°42′24″N/000°01′04″W, n = 9 signal and 30 virile crayfish) (Fig. 1). Upon capture, animals were transported individually to Cardiff University (UK), humanely euthanized by freezing at -80 °C and stored in ca. 95% molecular grade ethanol before transport to Charles University in Prague for further processing. For *A. astaci* screening, we harvested from each animal a section of tail fan, soft abdominal cuticle, two limb joints, and any melanised cuticle (as in Svoboda et al., 2014). Tissue samples from each individual (40–50 mg) were ground together in liquid nitrogen from which DNA was extracted using a DNeasy tissue kit (Qiagen) as per manufacturer's guidelines.

All samples were screened for *A. astaci* presence using TaqMan MGB quantitative PCR (qPCR) on the iQ5 BioRad thermal cycler according to Vrålstad et al. (2009), slightly modified to increase assay specificity (Strand et al., 2011; Svoboda et al., 2014). To check for potential inhibition (as in Kozubíková et al., 2011; Svoboda et al., 2014) each DNA isolate was analysed by qPCR at two concentrations (undiluted and 1:10 dilution). Negative controls were included at each step of the protocol, and in all cases these remained negative. Based on the estimated amount of *A. astaci* DNA in the isolates (expressed in PCR-forming units, PFU), the extent of the infection was expressed in semi-quantitative agent levels (A0-A7; according to Vrålstad et al., 2009; Kozubíková et al., 2011). Samples with agent levels of A2 or higher were considered positive for *A. astaci*.

For *A. astaci* genotype group identification, we analysed *A. astaci*-positive samples using nine *A. astaci*-specific microsatellite markers (Grandjean et al., 2014). As amplification success depends on the amount of pathogen DNA in the sample, genotyping was only attempted for those with agent level A3 and higher (as in Grandjean et al., 2014) and was repeated three times for each sample. In case of an initial lack of amplification, DNA isolates were concentrated on the Concentrator Plus 5305 (Eppendorf). The results of successful genotyping were compared with the *A. astaci* reference strains described by Grandjean et al. (2014) and an *A. astaci*-positive DNA isolate from signal crayfish in Lake Mochdre (Newtown) Wales, UK (James et al., 2017).

3. Results

Within allopatric sites on the River Lea, *Aphanomyces astaci* was detected in 83% (25 out of 30) signal crayfish but was not detected in any virile crayfish (n = 30). From the sympatric site, 44% (4 out of 9) signal crayfish and 23% (7 out of 30) virile crayfish tested positive for *A. astaci* infection. All *A. astaci*-positive samples yielded low levels of infection (A2-A3; Vrålstad et al., 2009). Of the *A. astaci* infected animals from the allopatric signal crayfish site A3 level infections were detected in four animals (estimated PFUs: 51, 71, 106 and 111). Within the sympatric site, A3 level infections were detected in three virile (estimated PFUs: 85, 167 and 1000) and two signal (estimated PFUs: 52 and 57) crayfish.

Due to low amount of *A. astaci* DNA, reliable amplification and scoring of the microsatellites were only possible for two specimens of virile crayfish. Of the nine microsatellite loci, amplification was

Fig. 1. Sample sites along the River Lea (UK) and an adjacent lake for invasive signal (*Pacifastacus leniusculus*) and virile (*Orconectes* cf. *virilis*) crayfish. River flow direction is indicated by the black arrow. Allopatric sites shown for signal (●) and virile crayfish (▲) respectively, and the sympatric site (■). Note: the location of the allopatric virile crayfish site is a lake adjacent to the river. More details about virile and signal crayfish distribution in this region can be found in James et al. (2016). Image courtesy of Maps data 2016 @Google.