A case of close interspecific interactions between diatoms: selective attachment on a benthic motile species

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Abstract :

Although diatoms are extensively used as bioindicators, positive interactions between species have been rarely investigated. In this study, the attachment of diatoms of the genus *Amphora* on benthic motile diatoms is described. The analysis of a river biofilm sample shows that the attached diatoms clearly select large sigmoid *Nitzschia* hosts. The examination of a regional database suggests that the attachment of *Amphora copulata* may be common in rivers and that the preferred host is *Nitzschia sigmaidea*.

Keywords : Amphora, Nitzschia, Bacillariophyta, bioindicators, niche

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The extensive use of diatoms as bioindicators of water quality assumes that the composition of diatom communities is largely driven by abiotic environmental filtering (Lobo et al. 2016). However, neutral processes and especially biotic interactions may also considerably shape microalgal communities (Bottin et al. 2016). The most studied interactions among diatom species are those mediated by competition for light and nutrients (Titman 1976). Cases of positive interactions between species have been rarely documented (e.g. Vanellander et al. 2009). Epiphytism generally implies a large difference in size between the epiphytes and their host. Diatoms are well known epiphytes on filamentous algae and aquatic macrophytes. However, the attachment of a diatom on another diatom is less common and has been rarely reported. In this study, we present the selective attachment of a small freshwater diatom species on another benthic motile species. This interaction was qualitatively described by Round and Lee (1989) from observations on glasses placed on top of sediment surfaces. We provide here a quantitative analysis from a local to a regional scale.

A biofilm sample was scraped in the Arc river (43°30'41.7''N, 5°28'57.2''E), France, on the flank of a large rock near the river bank. The fresh sample was observed under a light microscope. Diatoms were the most abundant microalgal group. Amongst a large amount of filaments of *Melosira varians*, many long and often sigmoid diatoms belonging to the genus *Nitzschia* were seen covered with a variable number of small diatoms (Fig. 1). The attached diatoms exhibit a cymbal shape when they are located on the edge of the host valve and have a biconvex outline when seen in the middle of the host valve. These observations typically correspond to the valve and girdle views of diatoms belonging to the genus *Amphora*. Nevertheless, it is difficult to identify species from fresh material (Cox 1996) and a sample digestion with boiling H₂O₂ (1 hour) is necessary to remove organic matter, and observe the specific ornamentation of the siliceous frustules. The attached diatoms and their host were separated during the treatment. The most abundant *Amphora* species (4.8 %) and the most likely to correspond to the high number of attached diatoms was *Amphora copulata* (Kützing) Schoeman & Archibald 1986 (ACOP) (Table 1). However, the attached diatoms may also belong to the less abundant *Amphora* species identified in the sample, such as *Amphora pediculus* (1 %) or *Amphora indistincta* (0.2 %).

Figure 1. Light microscope photographs. A frustule of *Nitzschia sigmoidea* (a). A cell of *N. sigmoidea* covered with *Amphora* sp. (b, c, d), two conspicuous droplets (arrows, d) are characteristics of *Amphora* and can be seen in some attached cells (d). Frustules of *N. sigmoidea* and *A. copulata* probably accidentally re-associated after sample H₂O₂ treatment (e). A single cell of *A. copulata* on a filament of *Melosira varians* (f). Scalebar = 10 µm

Table 1. List of taxa present in the H₂O₂-treated epilithic sample with estimation of their relative abundance (total abundance = 501).

Taxon	Relative abundance (%)
<i>Melosira varians</i> Agardh	48.1
<i>Gyrosigma sciotense</i> (Sullivan et Wormley) Cleve	9.0
<i>Amphora copulata</i> (Kützing) Schoeman & Archibald	4.8
<i>Navicula lanceolata</i> (Agardh) Ehrenberg	4.8
<i>Nitzschia recta</i> Hantzsch in Rabenhorst	4.4
<i>Nitzschia sociabilis</i> Hustedt	4.2
<i>Nitzschia dissipata</i> (Kützing) Grunow ssp. <i>dissipata</i>	2.6
<i>Navicula tripunctata</i> (O. F. Müller) Bory	2.6
<i>Nitzschia dubia</i> W. M. Smith	2.0
<i>Diatoma vulgaris</i> Bory	1.4
<i>Navicula gregaria</i> Donkin	1.4
<i>Amphora pediculus</i> (Kützing) Grunow	1.0

<i>Nitzschia vermicularis</i> (Kützing) Hantzsch in Rabenhorst	1.0
<i>Surirella brebissonii</i> Krammer & Lange-Bertalot var. <i>brebissonii</i>	1.0
<i>Nitzschia sigmoidea</i> (Nitzsch) W. Smith	0.8
<i>Nitzschia wuellerstorffii</i> Lange-Bertalot	0.8
<i>Frustulia vulgaris</i> (Thwaites) De Toni	0.6
<i>Gyrosigma attenuatum</i> (Kützing) Rabenhorst	0.6
<i>Navicula cryptotenella</i> Lange-Bertalot	0.6
<i>Nitzschia heufleriana</i> Grunow	0.6
<i>Nitzschia thermaloides</i> Hustedt	0.6
<i>Encyonema prostratum</i> (Berkeley) Kützing	0.4
<i>Navicula reichardtiana</i> Lange-Bertalot var. <i>reichardtiana</i>	0.4
<i>Tryblionella apiculata</i> Gregory	0.4
<i>Amphora indistincta</i> Levkov	0.2
<i>Cocconeis euglyptoides</i> (Geitler) Lange-Bertalot	0.2
<i>Caloneis lancettula</i> (Schulz) Lange-Bertalot & Witkowski	0.2
<i>Cocconeis pediculus</i> Ehrenberg	0.2
<i>Cymatopleura solea</i> (Brebisson in Breb. & Godey) W. Smith var. <i>solea</i>	0.2
<i>Diatoma vulgaris</i> Bory (abnormal form)	0.2
<i>Fallacia helensis</i> (Schulz) D.G. Mann	0.2
<i>Fallacia pygmaea</i> ssp. <i>subpygmaea</i> Lange-Bertalot Cavacini Tagliaventi & al	0.2
<i>Navicula antonii</i> Lange-Bertalot	0.2
<i>Nitzschia capitellata</i> Hustedt in A. Schmidt & al	0.2
<i>Nitzschia dubia</i> W.M. Smith (abnormal form)	0.2
<i>Nitzschia angustata</i> (W. Smith) Grunow	0.2
<i>Nitzschia intermedia</i> Hantzsch ex Cleve & Grunow	0.2
<i>Navicula kotschyi</i> Grunow	0.2
<i>Nitzschia linearis</i> (Agardh) W.M. Smith var. <i>linearis</i>	0.2
<i>Nitzschia paleacea</i> (Grunow) Grunow in van Heurck	0.2
<i>Nitzschia palea</i> (Kützing) W. Smith var. <i>palea</i>	0.2
<i>Navicula radiosa</i> Kützing	0.2
<i>Navicula rostellata</i> Kützing	0.2
<i>Navicula simulata</i> Manguin	0.2
<i>Navicula veneta</i> Kützing	0.2
<i>Nitzschia linearis</i> (Agardh) W. M. Smith var. <i>tenuis</i> (W. Smith) Grunow in Cleve	0.2
<i>Nitzschia supralitorea</i> Lange-Bertalot	0.2
<i>Rhoicosphenia abbreviata</i> (C. Agardh) Lange-Bertalot	0.2
<i>Reimeria uniseriata</i> Sala Guerrero & Ferrario	0.2
<i>Surirella amphioxys</i> W. Smith	0.2
<i>Surirella ovalis</i> Brébisson	0.2
<i>Stephanocostis chantaicus</i> Genkal & Kuzmin	0.2
<i>Tryblionella debilis</i> Arnott ex O'Meara	0.2
<i>Ulnaria ulna</i> (Nitzsch) Compère	0.2

The long sigmoid *Nitzschia* could be either *N. sigmaidea* or *N. vermicularis*, which had similar relative abundance in the sample. These species of *Nitzschia* are able to move on a substrate in curved directions. The *Nitzschia* observed in the river sample moved at a speed about $20 \mu\text{m.s}^{-1}$ which did not seem reduced when attached diatoms were present (S 1).

After observation of the live material, the sample was fixed with dilute lugol to stop cell movement before counting. There was no visible effect of fixation on cell associations. In order to assess the host specificity of the attached diatoms, five groups of abundant and large diatom forms were considered as potential hosts for ACOP (Table 2). The cells from each group, with and without attached diatoms, were counted under a light microscope at $\times 100$ magnification (Table 2). About two thirds of the long *Nitzschia* had attached diatoms. However, no epiphyte was seen on more than one hundred observed individuals of the genus *Gyrosigma*, which have several common characteristics with the *Nitzschia* species that were observed in the sample: a large size, a sigmoid shape and a similar motility. There were only five cases (3 %) of attachment on filaments of *Melosira varians* and in each case, only one cell of ACOP was visible on the filament (Fig. 1). No attachment was observed on any large *Navicula* species and neither on the other genera of large diatoms represented in the river sample. These results demonstrated a clear host selection.

Table 2. Number of diatom forms with and without attached diatoms, identified in the fresh biofilm sample (2 microscope slides were completely screened). The different forms listed in the table were assumed large enough to be potential hosts. In brackets are indicated the taxa representing each form which were identified after sample treatment with boiling H_2O_2 .

Potential host diatom forms	With attached diatoms	Without attached diatoms
Long <i>Nitzschia</i> ($>100 \mu\text{m}$, <i>N. sigmaidea</i> , <i>N. vermicularis</i> , ...)	39	20
<i>Gyrosigma</i> (<i>G. sciottense</i> and <i>G. attenuatum</i>)	0	105
Filaments of <i>Melosira varians</i>	5*	149
Large <i>Navicula</i> (<i>N. tripunctata</i> , <i>N. lanceolata</i> , <i>N. capitatoradiata</i> , ...)	0	94
Others ($>50 \mu\text{m}$, <i>Cymatopleura</i> , <i>Surirella</i> , <i>Nitzschia</i> , <i>Diatoma</i> , ...)	0	48

*In each case, only one cell was attached on the filament.

The significance of attachment of ACOP at larger spatial scale was assessed through the analysis of co-occurrences in a recently published regional database of species incidence and environmental conditions among 196 sites in western France (Bottin et al. 2016). ACOP was present in 44% of the samples in the regional database and in 86% of the samples in which *N. sigmaidea* occurred. Focus was given to the potential hosts observed in the Arc river sample: *N. sigmaidea* (in 11% of total samples), *N. vermicularis* (7%), *M. varians* (78%). *Cymatopleura solea* (13%) was also considered due to its presence in the river sample and the database, its large size, and because it presents fibulae on its frustule which may be a criterion of host selection by ACOP (Round & Lee 1989). Two hypotheses were tested. The first one assumes that there is no more co-occurrences of ACOP with its potential host in the

database than there may be by chance if the occurrences of ACOP were distributed randomly among sites. Therefore, the exact probability to get at least as many co-occurrences with ACOP as observed in the database was calculated from the number of possible combinations (Table 3, S 2). Also, random permutations ($n=9999$) of incidence data of ACOP across sites were carried out and the observed number of co-occurrences with each potential host species was compared with the distribution of simulated co-occurrences by permutations (Fig. 2). The co-occurrences of ACOP with *N. sigmoidea* and *C. solea* were significant ($p < 0.05$) whereas they were not significant with the two other species. Thus, there is no sign in the database of an association of ACOP with *N. vermicularis* or *M. varians*. This suggests that among the two *Nitzschia* species, *N. sigmoidea* may be a preferred host for ACOP and that attachment on *M. varians* is probably rare.

Figure 2. Distribution of the F ratio of observed over simulated co-occurrences of *Amphora copulata* with 4 potential host species. The vertical segments represent the lower 95 % of the distribution and the cross is the median. The results of permutations ($n=9999$) of *A. copulata*'s occurrences among sites are represented in grey. The black segments correspond to simulations of *A. copulata*'s occurrences using an environmental model (random forest) taking into account physico-chemical conditions at each site.

Table 3. Number of occurrences and co-occurrences with *Amphora copulata* of 4 potential host species among the 196 samples of a regional database (Bottin et al. 2016), and probability to obtain randomly at least as many co-occurrences under the random and the abiotic environmental-control hypothesis.

Diatom species	Occurrences	Co-occurrences with <i>A. copulata</i>	Probability of at least as many co-occurrences	
			random	environmental
<i>Amphora copulata</i>	87	-	-	-
<i>Nitzschia sigmaidea</i>	22	19	0.00002 ^a	0.00248 ^c
<i>Nitzschia vermicularis</i>	13	8	0.15891 ^a	-
<i>Melosira varians</i>	153	70	0.2975 ^b	-
<i>Cymatopleura solea</i>	25	17	0.00995 ^a	0.15617 ^c

^a exact probability, ^b estimated from permutations (n=9999), ^c estimated from random forests

The second hypothesis is that the occurrences of ACOP are only driven by abiotic environmental factors and are not favored by the presence of a potential host species (*N. sigmaidea* or *C. solea*). Therefore, as with permutations, new patterns of occurrences of ACOP among sites were simulated, taking this time into account the species' ecological preferences. Following Bottin *et al* (Bottin et al. 2016), random forests were used (R package 'randomForest', Liaw and Wiener (2002)) to predict the occurrence of ACOP from the sixteen abiotic environmental parameters describing each sample of the database (elevation, slope, distance to the source and water quality parameters). The presence of ACOP at the sites where a potential host was present, was predicted from a random forest (500 trees) trained with observed occurrences of ACOP at the sites where the potential host did not occur ($R^2 = 0.34$ with *N. sigmaidea* and 0.25 with *C. solea*). The obtained pseudoprobabilities of presence (prediction scores) at all sites were used to simulate $n=10000$ patterns of occurrence of ACOP by drawing for each site, n times in a Bernoulli law. They were also used to derive the probabilities to get at least as many co-occurrences as observed in the database (Table 3, S 2). Under this second hypothesis, the observed co-occurrences of ACOP with *C. solea* were not significant. However, the co-occurrences with *N. sigmaidea* were still significant ($p < 0.05$) and suggest that *N. sigmaidea* may facilitate the presence of ACOP by providing an adequate substrate for attachment. Nevertheless, the tests only show that the data are consistent with a common attachment of ACOP on *N. sigmaidea* in rivers but the extent of this phenomenon can only be demonstrated by microscopic observations. Moreover, the power of the tests is limited for several reasons. The main one is the difference in mean abundance of ACOP and *N. sigmaidea* across sites which results from the size-abundance ecological relationship (White et al. (2007), 6:1 ratio in our sample), whereas the counting effort is usually constant among samples. Co-occurrences of the two species may be therefore underestimated. Thus, it cannot be concluded from the high proportion of sites where ACOP is present but *N. sigmaidea* is not ($39\% = (87-19)/(196-22)$, Table 3), that ACOP may have other hosts or other habitats.

This case of attachment is particularly interesting because it involves a large number of diatoms on the frustule of a single motile diatom (Fig. 1) which can move in spite of the numerous attached cells. Moreover, this attachment is selective and probably widespread as the co-occurrences of ACOP and *N. sigmaidea* in a regional database suggest. ACOP may

benefit from this interaction because the host moves relatively fast and can transport it where light and nutrients are available (Cohn & Disparti 1994, Bondoc et al. 2016). This can be at the expense of *N. sigmoidea* which may suffer higher competition for resources with attached cells, undergo stronger drag and increased downstream drift, and be less mobile in dense biofilms. Ecophysiological studies on the association of the two species are needed to check the possibility that this relation may be an epiphytism, a parasitism or even a mutualism, if benefits for *N. sigmoidea* could be demonstrated. More generally, there is a need to clarify positive interactions between species for a more accurate use of diatoms as bioindicators in neo- or paleolimnology. Indeed, if the presence of a species is more determined by biotic interactions than by water quality, this species will have a poor indicator value. Moreover, for paleo-environmental reconstructions, it makes a difference whether a species is epiphytic on macrophytes or on epilithic diatoms.

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S 1. Video sequence made from the river Arc sample divided into three parts: (a) movement of a 120 µm-long *Nitzschia* species, (b) same species with two attached *Amphora* cells. The cell swings from connective to valve side without slowing down (LM ×100). (c) *Nitzschia sigmoidea* moving with several *Amphora* cells attached to its frustule (LM ×200) (Material: Leica DFC290 hd camera on a Leica DM5500B microscope, frame rate=30/s).

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S 2. Calculation of conditional probabilities given the observed occurrences of a potential host species in the regional database.

C = number of co-occurrences of ACOP and a potential host

n = number of sampled sites

n₁ = number of occurrences of ACOP

n₂ = number of occurrences of a potential host

$$\text{Random hypothesis: } p(C \geq Co) = \sum_{k=Co}^{n_2} \frac{C_{n_2}^k \cdot C_{n-n_2}^{n_1-k}}{C_n^{n_1}}$$

$$\text{Environmental model: } p(C \geq Co) = \sum_{k=Co}^{n_2} \sum_{X \in P_k} \prod_{i \in X} p_i \prod_{j \in \bar{X}} (1 - p_j)$$

where P_k are the possible combinations of k sites among the sites where a potential host is present, p_i is the random forest-derived pseudo-probability of occurrence of ACOP at site i .