Received: 6 October 2021 | Revised: 3 December 2021 | Accepted: 3 December 2021

DOI: 10.1111/mec.16330

NEWS AND VIEWS

PERSPECTIVE

MOLECULAR ECOLOGY WILEY

Searching for transmissible cancers among the mussels of Europe

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Transmissible cancers are infectious malignant cell clones that spread among individuals through transfer of living cancer cells. Several such clones have been identified in various species of marine bivalve molluscs, including mussels, clams and cockles. These parasitic cell lineages cause a leukaemia-like disease called disseminated neoplasia, and are presumed to pass between hosts by ingestion of waterborne cancer cells during filter feeding. Although occasional cases of transmissible cancer had previously been identified in mussels of the genus *Mytilus* in Europe, the number of distinct clones affecting these animals, and their prevalence, was unknown. In this issue of *Molecular Ecology*, Hammel et al. (2021, 30) present findings from a large-scale screen for transmissible cancer across 5907 European *Mytilus* mussels. Using a genotyping approach, Hammel et al. searched for signal of genetic chimerism, which can arise due to infection by transmissible cancer cells. The screen detected a previously identified globally distributed mussel transmissible cancer at very low prevalence, and found no evidence of additional contagious clones. A parallel histological screen additionally revealed low prevalence of a nontransmissible form of disseminated neoplasia. By quantifying the burden of disseminated neoplasia in European mussel populations, this study provides strong foundations for future work investigating the origins, evolution and impacts of transmissible cancers in mussels.

Disseminated neoplasia is a leukaemia-like disease of marine bivalve molluscs characterised by the presence of excessive numbers of abnormal proliferative round cells. These occupy body cavities and infiltrate tissues, often causing death (Carballal et al., 2015). The condition was first described in oysters in the 1960s, and was subsequently recognised in other bivalve species. Its tendency to occur in outbreaks linked to mass mortality events in some species led to speculation that the disease might be associated with an infectious agent, possibly a virus (Carballal et al., 2015).

Work by Metzger et al. (2015, 2016) demonstrated that some forms of disseminated neoplasia are indeed contagious, but the identity of the infectious agent was altogether unexpected: transmissible cancer. Such contagious clonal cell lineages had until then been described only in two distantly related mammals, dogs and Tasmanian devils (Murchison, 2009). Their discovery in bivalves suggested that this peculiar disease process was more widespread in nature than previously appreciated.

The evidence that at least some cases of bivalve disseminated neoplasia were caused by transmissible cancer was indisputable. Transmissible cancers do not derive from their hosts' own tissues, but instead carry the DNA of the original "founder animal" whose somatic cells spawned the cancer in the first place (Figure 1). Thus, individuals hosting transmissible cancers are chimeras, identifiable as two genetically distinct populations of cells co-existing within the same body. Further, apart from a small subset of mutations acquired while in the cancerous state, cells belonging to a common transmissible cancer lineage are genetically identical. This implies that cancers occurring in different animals would be expected to show the same DNA fingerprint. Genetic analysis of disseminated neoplasia in several species had revealed this distinctive pattern of clonality

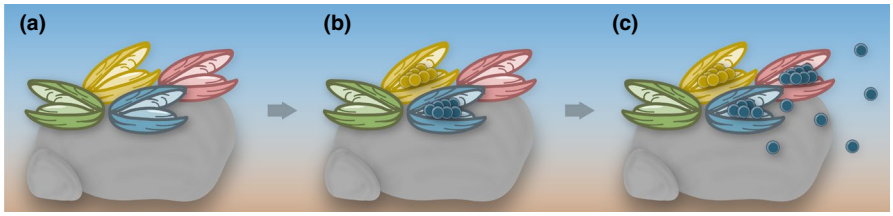

FIGURE 1 Transmissible cancers produce signals of genetic chimerism. (a) Marine bivalve molluscs, such as mussels, often occur in colonies. (b) Disseminated neoplasia (represented by coloured round cells) is a leukaemia-like cancer that arises sporadically in individual animals. Genetic analysis of these sporadic cancers reveals genetic identity between cancer and host (animals and cancer cells in same colour). (c) Occasionally, malignant cells from disseminated neoplasia disperse through seawater and infect other animals. In such cases of transmissible cancer, infected hosts and cancer cells carry different genotypes, which is detectable as genetic chimerism (animal and cancer cells in different colour)

FIGURE 2 Mussel beds in Brittany, France, found to be affected by transmissible cancer

and chimerism, confirming the presence of transmissible cancer; to date, seven such contagious cell lineages have been discovered in bivalves (Garcia-Souto et al., 2021; Metzger et al., 2015, 2016; Skazina, Odintsova, Maiorova, Ivanova, et al., 2021; Yonemitsu et al., 2019). Importantly, however, it appeared that not all cases of disseminated neoplasia were associated with transmissible cancer, as some affected animals lacked this signal, instead showing genetic profiles consistent with the presence of ordinary cancer arising through transformation of the diseased individual's own cells.

Among the bivalve species confirmed to host transmissible cancers were marine mussels of the *Mytilus edulis* complex. This widespread group includes six incompletely reproductively isolated species, each with broad geographical ranges. Two species, *M. edulis* and *M. galloprovincialis*, as well as their hybrids, are the primary members of the complex inhabiting the coasts of Europe (Figure 2). Disseminated neoplasia had been reported in both species, with prevalence ranging from less than 1% to more than 50% (Benadelmouna et al., 2018; Burioli et al., 2019; Carballal et al., 2015; Charles et al., 2020). At least some of these cases were known to be attributable to one or more transmissible cancer clones derived from a third member of the species complex, *M. trossulus*, but the frequency and distribution of these were unknown (Burioli et al., 2019; Riquet et al., 2017; Yonemitsu et al., 2019). Moreover, the 2010s brought increased interest in the diseases of marine mussels, after unexplained mass mortality events reduced populations in northern France (Benadelmouna et al., 2018; Charles et al., 2020). Hammel et al. set out to systematically assess the prevalence of transmissible cancer in the *M. edulis* complex of mussels in Europe.

The authors sampled 5907 *Mytilus* mussels (*M. edulis*, *M. galloprovincialis* and their hybrids) collected at more than two hundred European coastal locations between 2005 and 2019, and used a fluorescence-based assay to genotype these animals at 106 loci. The team devised three metrics to screen the data for genetic anomalies consistent with chimerism. First, they searched for individuals with an unusually high proportion of "uncalled" alleles. In normal diploid individuals, the fluorescence-assigned genotype at each locus usually falls into one of three easily recognisable states: homozygous reference, homozygous alternative, or heterozygous. Fluorescence signals from chimeric individuals, however, are complex and reflect the genotypes of the two contributing cell populations and their relative fractions within the sample. Loci with unexpected fluorescence were classified as "uncalled". Second, in 1749 individuals from which two different tissues were sampled, the authors searched for subtle differences in fluorescence signal between the matched pair, reasoning that variation in genotype might reflect differing transmissible cancer cell fractions. Finally, as the previously discovered transmissible cancers in *Mytilus* species were derived from *M. trossulus*, the team searched for individuals showing a high proportion of alleles considered diagnostic for *M. trossulus*.

The screen detected genetic chimerism attributable to transmissible cancer in European mussels at very low prevalence (25/5907 samples, 0.42%). Although the method may have lacked sensitivity to detect low-level disease, and hence prevalence may have been somewhat underestimated, the results suggest that transmissible cancer is unlikely to have been responsible for French mussel die-offs, consistent with other studies (Charles et al., 2020). Cases were found in both *M. edulis* and *M. galloprovincialis*, as well as in their hybrids. Remarkably, however, the cancers in all 25 infected individuals were of *M. trossulus* origin. Further investigations indicated that these almost certainly all belonged to a single globally distributed transmissible cancer clone, known as *M. trossulus* bivalve transmissible neoplasia 2 (MtrBTN2), which had been previously detected in European *M. edulis* (Yonemitsu et al., 2019). This result adds to the collection of *M. edulis* complex species known to host MtrBTN2, which also includes *M. trossulus* itself, sampled in the Sea of Japan and the Sea of Okhotsk, as well

as the South American species, *M. chilensis* (Skazina, Odintsova, Maiorova, Frolova, et al., 2021; Skazina, Odintsova, Maiorova, Ivanova, et al., 2021; Yonemitsu et al., 2019). Thus, MtrBTN2 is a single cancer lineage, which first arose from the malignant cells of an individual *M. trossulus* "founder animal", and has subsequently spread around the world through transmission of its cancer cells between mussels. The mode whereby MtrBTN2 has covered such vast distances is unknown, although it seems probable that inadvertent transport of diseased animals adhered to shipping vessels has played a role (Yonemitsu et al., 2019).

The genetic chimerism screen developed by Hammel et al. was a useful tool for the detection of transmissible cancers, but was not equipped to detect cancers that arose from the bodies of their hosts. In order to estimate the prevalence of such nontransmissible forms of disseminated neoplasia in European *Mytilus* mussels, the authors performed histological assessment of 222 animals, discovering eight cases (3.6%) of nontransmissible cancer. Unexpectedly, the affected mussels were all sampled on the same date at the same location, and their cancers showed distinctive and similar morphological features. This raises the possibility that these cancers, although host-derived, shared genetic, environmental, or even infectious risk factors that contributed to their development.

The findings reported by Hammel et al. (2021) join a body of evidence suggesting that spontaneous disseminated neoplasia may not be uncommon in some marine bivalve molluscs (Burioli et al., 2019; Metzger et al., 2016; Yonemitsu et al., 2019). However, it appears that very few of these cancers become widespread transmissible clones. We do not yet know whether all cases of disseminated neoplasia are fully equipped to become contagious, or if, instead, the ability to spread from host to host requires a particular precursor cell state or programme of genetic changes. There are rich avenues for future research examining the mechanisms of transmission and the role of immunity in preventing infection. Some transmissible cancers, including MtrBTN2, are capable of infecting animals of closely related species beyond their original host species; the determinants of species specificity are, however, still unclear. Furthermore, bivalve transmissible cancer clones appear to show different epidemiological patterns. Whereas some may occur at high frequency in populations and are probably associated with mass mortality events (Metzger et al., 2015, 2016), others, such as MtrBTN2, have been observed at low prevalence (Hammel et al., 2021; Skazina, Odintsova, Maiorova, Ivanova, et al., 2021; Skazina, Odintsova, Maiorova, Frolova, et al., 2021). Cancer cell traits influencing transmissibility, together with host immunological and ecological factors, are likely to contribute to these varied dynamics.

Once established as transmissible cancers, how long can these bizarre underwater parasitic clones persist? The sexually transmitted contagious cancer affecting dogs first arose several thousand years ago, indicating that some transmissible cell lineages can survive for extended periods (Murchison, 2009). We do not yet know the age of MtrBTN2, or of the other bivalve transmissible cancer lineages. Analysis of the genetic variants captured in these clones' DNA may eventually permit estimation of mutation rates and, through extrapolation, dates of origin. Such work may also illuminate phylogenetic relationships among cancer samples collected from different animals, as already hinted by Hammel et al., perhaps revealing geographical routes of disease spread.

Hammel et al. present an ingenious low-cost method for genetic surveillance of transmissible cancers in bivalves and apply this, with intriguing results, to a large collection of *Mytilus* mussels. Transmissible cancers are a newly recognised pathogen of bivalve molluscs, whose distribution and impact remain poorly understood. By charting their prevalence among European mussels, this study paves the way towards further understanding of the origins and evolution of these elusive infectious cell lineages.

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How to cite this article: Baez-Ortega, A., & Murchison, E. P. (2021). Searching for transmissible cancers among the mussels of Europe. *Molecular Ecology*, 00, 1–4. https://doi.org/10.1111/mec.16330