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From fish to frogs and beyond: Impact and host range of emergent ranaviruses

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ABSTRACT

Ranaviruses are pathogens of ectothermic vertebrates, including amphibians. We reviewed patterns of host range and virulence of ranaviruses in the context of virus genotype and postulate that patterns reflect significant variation in the historical and current host range of three groups of *Ranavirus*: FV3-like, CMTV-like and ATV-like ranaviruses. Our synthesis supports previous hypotheses about host range and jumps: FV3s are amphibian specialists, while ATVs are predominantly fish specialists that switched once to caudate amphibians. The most recent common ancestor of CMTV-like ranaviruses and FV3-like forms appears to have infected amphibians but CMTV-like ranaviruses may circulate in both amphibian and fish communities independently. While these hypotheses are speculative, we hope that ongoing efforts to describe ranavirus genetics, increased surveillance of host species and targeted experimental assays of susceptibility to infection and/or disease will facilitate better tests of the importance of hypothetical evolutionary drivers of ranavirus virulence and host range.

1. Background

Ranaviruses are large double-stranded DNA viruses of the family *Iridoviridae*, which infect amphibians, reptiles and fish (Duffus et al., 2015). They are considered important emergent pathogens and several lines of evidence point to humans playing a significant role in emergence: 1) disease outbreaks have occurred frequently in cultured amphibians and fish (Zhang et al., 2001), 2) ranavirus has been detected frequently infecting invasive populations of non-native species and traded animals in Europe, Asia and South America (Une et al., 2009; Sharifian-Fard et al., 2011; Soto-Azat et al., 2016) and 3) translocations by humans are thought to have facilitated range expansion on at least two continents (Picco and Collins, 2008; Price et al., 2016). The broad host range and role of people in emergence were key reasons behind the World Organisation for Animal Health's (OIE) decision to list ranaviruses as notifiable pathogens of amphibians (Schloegel et al., 2010) and fish (OIE, 2016). However, the ecological impacts of emergent ranaviruses on their ectothermic hosts, at the levels of individuals and populations, are highly variable. In some cases, emergent disease causes extensive mortality and drives host populations into rapid demographic decline that can persist over multiple host generations. Alternatively, persistent disease dynamics may not be associated with observable population decline, and asymptomatic infections can also occur at high prevalence. Why host responses exhibit such variation, even within single host species is a subject of much debate and research effort.

Several ecological factors have been identified as correlates of mortality events. These factors can be broadly classified as: 1) age-dependent and host-specific susceptibility; 2) abiotic drivers, and; 3) host abundance and/or density. The difficulty with attribution is that many of these factors manifest coincidentally (Brunner et al., 2015). For example, outbreaks of *Epizootic haematopoietic necrosis virus* (EHNV) in red-finned perch (*Perca fluviatilis*) are age-specific and seasonal (Whittington et al., 2010), and mass mortality of adult common frogs may be linked to aggregation during breeding (Cunningham et al., 1996; Price et al., 2016). Attempting to completely disentangle the role of each factor is challenging due to interactions; temperature, for instance, can directly affect the outcome of ranavirus infections but also affects key aspects of the ecology of ectothermic vertebrates (Brunner et al., 2015).

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What is less explored is how systematic and pairwise variation in ranavirus genomes correlates with patterns of epidemic disease dynamics. However, research on *Frog virus 3* (FV3) has illustrated how the disease process is regulated by viral gene expression, ranaviruses evolve rapidly in single host species populations, and they recombine (Abrams et al., 2013; Epstein and Storfer, 2016; Price, 2016; Claytor et al., 2017). These findings, combined with evidence of heritable variation in host immunity to ranaviruses (Teacher et al., 2009; Echaubard et al., 2016), suggest that the emergence of epidemic disease dynamics (or lack thereof) must be governed to some degree by the genetic tools available to emergent ranaviruses. The specifics of gene conservation, expression and virulence are covered elsewhere, including in articles in this special issue. Here we review reports of ranavirus infection and epidemics in North and Central America, the region where ranavirus has been studied most intensively, and Europe, a region where a recently described ranavirus lineage is the cause of emerging disease, in the context of the phylogenetic identity of the causative ranaviruses. Where possible, we explore the relationships between ecological conditions and viral identity and comment on relative host ranges.

For our purposes, we will use terminology based on the phylogenetic analyses published in Jancovich et al. (2015), which identified four to five distinct *Ranavirus* lineages, but we will focus on the three associated with amphibians (frequently termed "amphibian-like ranaviruses" but hereon referred to as amphibian-associated ranaviruses): FV3-like ranaviruses, their sister group the common midwife toad virus (CMTV)-like variants, and the more basal *Ambystoma tigrinum virus* (ATV)-like group. These first two are monophyletic groups but for the purposes of this article we expand the ATV-like group to include all fish-associated forms at the base of the amphibian-like ranavirus phylogeny (Ariel et al., 2016; Subramaniam et al., 2016) which results in a paraphyletic group (Fig. 1). While there is significant variation encapsulated within these lineages (Echaubard et al., 2014), the deeper divisions described by these groups likely represent evolutionary steps that involved changes in host range germane to our topic (Jancovich et al., 2010; Abrams et al., 2013). As more complete ranavirus genomes become available, we expect that ranavirus taxonomy and systematics will undergo further revision.

2. FV3-like ranaviruses in American herpetofauna

Fifty years after the serendipitous discovery of FV3 in the United States, FV3-like ranaviruses continue to cause mortality across the planet in wild and captive amphibians, chelonians, fish and squamate reptiles (Granoff et al., 1965; Duffus et al., 2015). The early work on FV3 presaged several interesting aspects of the biology of this virus. First, while experiments with this and closely related ranaviruses were often lethal to larval, and to a lesser extent adult amphibians, some individuals survived with persistent, asymptomatic infections (Clark et al., 1968, 1969; Tweedell and Granoff, 1968; Wolf et al., 1968). Second, the first FV3-like ranaviruses were isolated from animals purchased from biological suppliers (Clark et al., 1969), although there are few details of their particular origins (see Granoff et al., 1965). Third, it became clear, at least from cell culture experiments, that FV3 and related viruses have very broad host ranges (Granoff et al., 1966; Clark et al., 1968). Each of these patterns has been upheld in the five decades since.

Ranaviruses have been detected in amphibians across the United States and Canada (Fig. 2; Duffus et al., 2015). The vast majority of ranavirus detections have been FV3-like ranaviruses associated with mortality events, especially in larval amphibians (Green et al., 2002; Miller et al., 2011; Duffus et al., 2015). North American FV3-like ranaviruses have thus developed a reputation for high virulence, which has been supported by laboratory infection experiments (e.g., Pearman and Garner, 2005; Schock et al., 2008; Echaubard et al., 2016), though the outcome of exposure varies a great deal with host phylogeny and life history correlates, and virus genotype (Hoverman et al., 2010, 2011). Whilst episodic and recurrent mass mortality events attributed

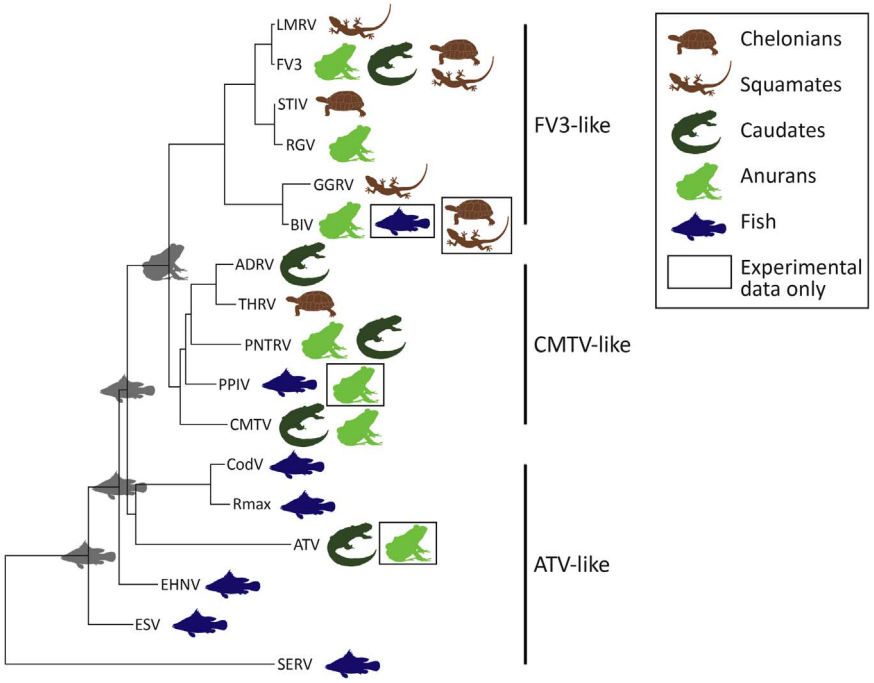

Fig. 1. Phylogenetic perspective on host range of ranaviruses in the context of broad virus type (*Frog virus* (FV)*3*-like, common midwife toad virus (CMTV)-like, *Ambystoma tigrinum virus* (ATV)-like). Putative ancestral hosts (fish or amphibian) are denoted by gray host images at nodes. Host ranges of individual isolates serve as a guide only and do not control for observer effort. Hosts are summarised based on membership of five higher order taxonomic groups: chelonian, squamate, caudate, anuran and fish. The overall topology of the tree follows Fig. 3 of Stohr et al. (2007), which was simplified by removing tips for clarity of presentation. Isolate abbreviations: SERV, short-finned eel ranavirus; ESV, European sheatfish virus; EHNV, *Epizootic haematopoietic necrosis virus*; ATV, *Ambystoma tigrinum virus*; Rmax, Ranavirus maximus; CodV, Cod iridovirus; CMTV, common midwife toad virus; PPIV, pike-perch iridovirus; PNTRV, Portuguese newt and toad ranavirus; THRV, *Testudo hermanni* ranavirus; ADRV, *Andrias davidianus* ranavirus; BIV, Bohle iridovirus; GGRV, German gecko ranavirus; RGV, *Rana grylio* iridovirus; STIV, Soft-shelled turtle iridovirus; FV3, *Frog virus 3*; LMRV, *Lacerta monticola* ranavirus.

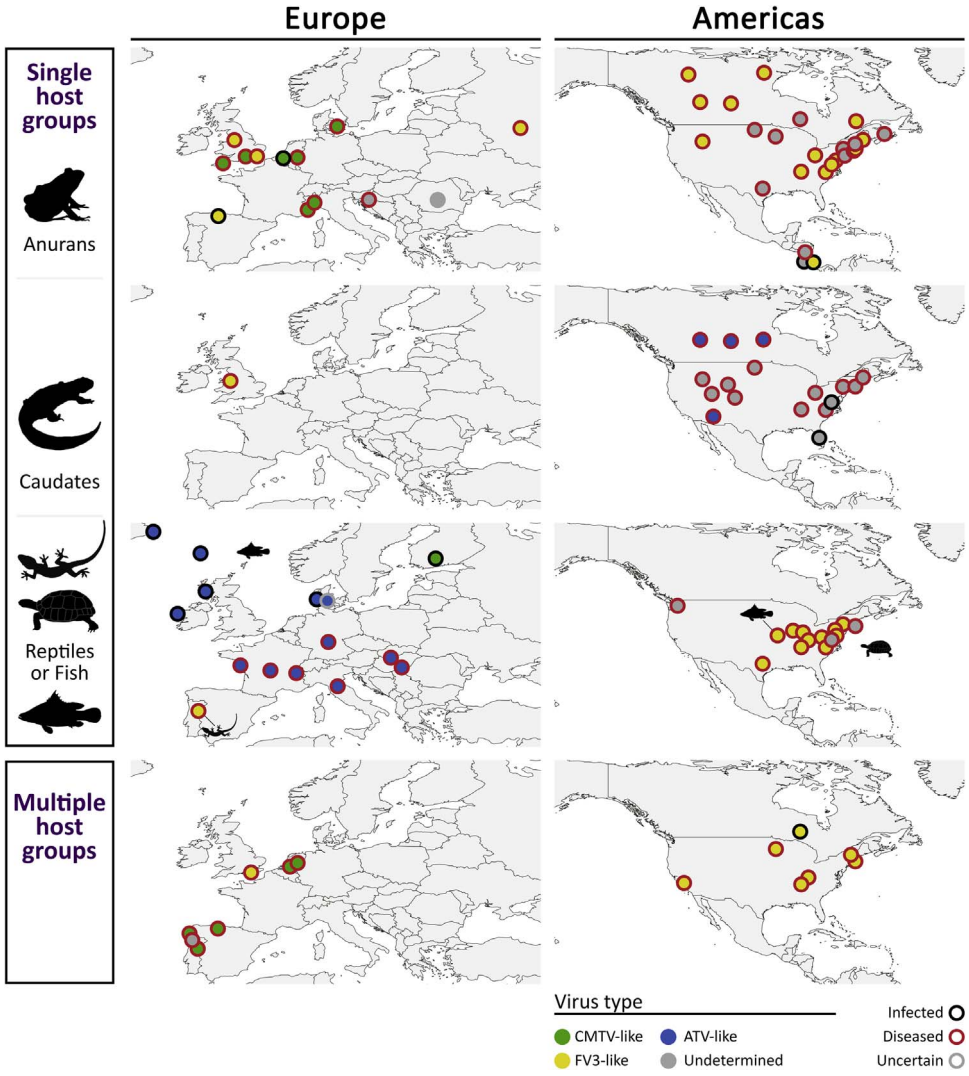

Fig. 2. Host range, distribution and impact of the major groups of amphibian-associated ranavirus in Europe and the Americas. Points mark approximate locations and sometimes represent multiple neighbouring incidents at a local scale which share the same attributes but could not be represented clearly as individual points at the continental scale used here. Datasets were generated by extending data tables published by Duffus et al. (2015).

to FV3-like ranaviruses have been noted in numerous amphibian species across North America and in all post-hatching life history stages, no clear case of population decline has been reported (Brunner et al., 2015). In addition, improved diagnostic methods and increased attention may explain the growing number of cases of FV3-like ranaviruses detected in wild populations in the absence of disease or notable mortality events (e.g., Crespi et al., 2015; O'Connor et al., 2016). Caution is warranted when interpreting an absence of reports of declines associated with ranavirosis, as lack of disease detection could be a consequence of sampling design (Gray et al., 2015). Outbreaks of ranavirosis and mortality events can last for less than two weeks and may go unobserved when monitoring is infrequent (Wheelwright et al., 2014; Hall et al., 2016). Few longitudinal studies at sites with recurring outbreaks have been conducted in North America (Brunner et al., 2015; Gray et al., 2015) Simulations indicate that local extinction of amphibian populations, especially isolated ones, is possible for both common and rare North American amphibian species (Earl and Gray, 2014; Earl et al., 2016).

Wild and captive chelonians and squamate reptiles infected with FV3-like viruses have also experienced lethal ranavirosis (Belzer and Seiber, 2011; Duffus et al., 2015; Kimble et al., 2015). Serological and PCR data in chelonians in North America reflect low incidence of infection which are short-lived because of high virulence (Johnson et al., 2010; Allender et al., 2013a, 2013b) or because they are quickly cleared (Brunner et al., 2015). Ranavirus infections in chelonians are likely, at least initially, the result of spill over from amphibian mortality events (e.g., Belzer and Seiber, 2011). Similarly, ranavirus infection in wild North American fish attributable to an FV3-like variant are rare, and one appears to have arisen through intimate contact with infected amphibians (Mao et al., 1999; Waltzek et al., 2014). Overall it appears that FV3-like ranaviruses predominantly exploit amphibian hosts in North America, can spill over into other vertebrate classes (Brenes et al., 2014), but may not be sustained when spill-over occurs.

Evidence is starting to accrue that FV3-like ranaviruses infect an incredibly broad range of ectothermic hosts in the rest of the Americas (Fox et al., 2006; Whitfield et al., 2013; Stark et al., 2014). For example, surveys of adult, Costa Rican amphibians report relatively low prevalence (16.6%) across a wide range of amphibian species but no mortality events have been detected (Whitfield et al., 2013). Conversely, the only record from Nicaragua is of a mortality event affecting amphibian larvae of several species (Stark et al., 2014). Preliminary molecular data shows high sequence identity to FV3 (Whitfield et al., 2012), but for both countries it is still unclear whether FV3-like ranaviruses are endemic or introduced.

Experimental data and case studies of captive and cultured ectotherms indicate that what is observed in the wild represents a

subset of available North American hosts susceptible to infection (and in many cases lethal ranavirosis) caused by FV3-like ranaviruses (Johnson et al., 2007; Miller et al., 2007; Hoverman et al., 2011; Waltzek et al., 2014). A lack of monitoring or infrequent sampling are likely to bias data on host and geographic ranges of ranaviruses, but host ecology and environmental conditions may also limit opportunity for transmission between hosts. Experimental data has also indicated that FV3-like ranaviruses are not equivalent (Hoverman et al., 2011; Duffus et al., 2014a); they may develop non-overlapping host specificities though environmental conditions will sometimes overwhelm the signature of host specialisation (Duffus et al., 2014a; Echaubard et al., 2014; Brand et al., 2016).

3. ATV-like ranaviruses in North American ambystomatid salamanders

FV3-like ranaviruses share North America with ATV-like forms (Fig. 2), initially described as the cause of recurrent and annual mass mortality of larval tiger salamanders (Jancovich et al., 1997; Docherty et al., 2003). While these mortality events can be catastrophic, affecting an entire year class, ATV epidemics do not always lead to notable mortality (Greer et al., 2009) and ATV has not been clearly linked to population declines (Brunner et al., 2004). As with FV3-like viruses, host populations vary in their resistance or tolerance to ATV infection (Schock et al., 2009) and ATV strains differ in their virulence (Brunner and Collins, 2009).

Initial experimental data supported the hypothesis that ATV is a caudate amphibian specialist however subsequent studies have shown that some anurans are susceptible to infection and lethal disease (Jancovich et al., 2001; Schock et al., 2008). Still, ATV has yet to be isolated from any hosts other than ambystomatid salamanders in the western half of North America, where they have an apparently long evolutionary history (Jancovich et al., 2005; Storfer et al., 2007; Epstein and Storfer, 2016). ATVs have yet to be shown to infect reptile or fish hosts, although sister taxa within the ATV-like group are fish pathogens (see below and Fig. 1; Jancovich et al., 2015; Stöhr et al., 2015). As with FV3-like ranaviruses, it is highly unlikely that the overall host range of ATV-like ranaviruses has been described exhaustively, but it is likely that subsequent research will continue to support a much narrower host range for North American ATVs compared to their FV3-like neighbours.

4. European fish infections due to two *Ranavirus* lineages

For four decades ranaviruses have been described infecting both marine and freshwater European fishes (Fig. 2; Jensen et al., 1979; Ahne et al., 1989; Ariel et al., 2010). Recent sequencing efforts support the conclusion that European piscine ranaviruses fall into two lineages: CMTV-like and our expanded classification of the proposed ATV-like ranaviruses (Jancovich et al., 2015; Ariel et al., 2016; Holopainen et al., 2016; Subramaniam et al., 2016). Arguably the most notable Ranavirus affecting fish is EHNV, individually listed as notifiable by the OIE and the European Union (Commission Directive 2008/53/EC). Although endemic in Australian redfin perch (*Perca fluviatilis*), EHNV has yet to be detected in Europe despite active surveillance by European Member and Associated States. Short-finned eel ranavirus (SERV) is another ATV-like ranavirus that appears to originate from Australasia but was opportunistically isolated once in Europe from non-native and asymptomatic eels imported from New Zealand (Bovo et al., 1999; Jensen et al., 2009). Ranavirosis in Europe was first described in marine fishes infected with an ATV-like ranavirus (Jensen et al., 1979), and subsequent surveys of aqua-cultured marine fish identified a near-identical virus infecting clinically healthy turbot fry (*Scophthalmus maxima*) and lumpfish (*Cyclopterus lumpus*) (Ariel et al., 2010, 2016; Stagg et al., 2017).

Wild European freshwater fish have experienced lethal ranavirosis attributable to ATV-like ranaviruses, the *European catfish virus* (ECV) and European sheatfish virus (ESV), which appear to be the same virus (Ahne et al., 1989; Marsh et al., 2002; Bigarre et al., 2008; Stöhr et al., 2015; Price, 2016). Although SERV has not been detected in wild or cultured European fish populations, experimental evidence showed that European freshwater species experience high levels of mortality when exposed to this ranavirus (Jensen et al., 2009), while North American bullheads (*Ameiurus nebulosus*) do not (Gobbo et al., 2010). Experimental studies also indicate that ECV has an even broader range of European fish hosts (Gobbo et al., 2010; Jensen et al., 2009, 2011). However common frogs (*Rana temporaria*) appear resistant to ECV, EHNV and SERV despite susceptibility to infection and disease caused by ranaviruses from two other lineages circulating in Europe (Bayley et al., 2013; Price et al., 2014). Although data on susceptibility of reptilian and amphibian hosts to these ATV-like ranaviruses isolated from fish is limited, none of these basal forms have been identified from either of these host groups and have therefore been postulated to be fish specialists (Ariel et al., 2016). Jancovich et al. (2010) proposed two models for ancestral host use in the amphibian-associated ranaviruses. The first involved an ancestral fish host and multiple, subsequent jumps into amphibians whilst the alternative involved an ancestral amphibian host which jumped into fish. Our synthesis supports an ancestral fish host (Jancovich et al.'s first model) and the classification of the ATV-like ranaviruses as a group of specialised fish viruses with ATV itself representing a single case of a member of the ATV-like group jumping between ectothermic host classes with subsequent adaptation and specialisation to ambystomatid salamanders (Fig. 1). In contrast, pike-perch iridovirus (PPIV) appears less phylogenetically constrained in host range. Opportunistically isolated from asymptomatic pike-perch fingerlings (*Lucio perca*), experiments have shown PPIV can infect and in some cases cause disease in other freshwater fish, but can also infect and cause lethal disease in common frogs (Tapiovaara et al., 1998; Jensen et al., 2009, 2011; Bayley et al., 2013). Whole genome sequence analysis of PPIV indicates it is CMTV-like (Holopainen et al., 2016), a clade increasingly recognized as important pathogens of amphibians and reptiles (see below).

5. Invasive FV3-like ranaviruses specialized for British common frogs

Epidemic ranavirosis affecting European amphibians that was first detected in the UK in the late 1980s (Cunningham et al., 1996) was caused by FV3-like forms that may have emerged twice in mainland Great Britain (Price et al., 2016). Ranavirus has yet to be detected on any other British islands, or on mainland Ireland (Price et al., 2016). All evidence therefore points to FV3 being recently (several decades) invasive in Britain. Although the exact route of introduction is unknown, human population density best explains the pattern of range expansion exhibited by British ranaviruses, which is a strong indication that spread is in part due to human activities (Price et al., 2016). Several aquatic vertebrate species commonly introduced to garden ponds preceding and during the emergence of UK ranavirosis, including Asian newts, North American bullfrogs, ornamental goldfish and carp, have been hypothesized as potential vectors (e.g. Hyatt et al., 2000). However, koi carp and goldfish were experimentally resistant to infection with FV3-like ranaviruses, and a ranavirus isolated from diseased koi in Asia is phylogenetically distinct from the FV3-like ranaviruses found in Great Britain (Bang Jensen et al., 2011; George et al., 2015). Ranavirus infections have yet to be described for Asian newts leaving the North American bullfrog, and North America, as a likely source of UK FV3-like ranaviruses.

North American FV3-like ranaviruses are notable for their host promiscuity, but host range of invasive British FV3-like ranaviruses appears to be comparatively narrow. FV3-like ranaviruses are responsible for mass mortality that has caused persistent local population declines that pose a conservation threat to common frogs (*R. tempor-*

aria) in England (Teacher et al., 2010). However, to date, incidents of mass mortality have predominantly affected adult common frogs with records of disease and mortality affecting other species comparatively rare. In addition, surveys of other common frog life history stages at locations where disease persists in adults have not revealed infections other than in adults (Duffus et al., 2013). Field data indicate that infection and disease in other British amphibian species is absent, geographically constrained (e.g., a single point location for the non-native and invasive common midwife toad, *Alytes obstetricans*) or in the case of the common toad (*Bufo bufo*), rare and unlikely to lead to significant levels of mortality or host population declines (Duffus et al., 2014a, 2014b). The comparatively reduced susceptibility of common toads compared to common frogs has been confirmed experimentally (Duffus et al., 2014a). Analyses of citizen science records of common frog mortality events even suggest that the presence of toads within amphibian potential host assemblages may be of benefit to frogs; whilst toads increased the likelihood of the occurrence of outbreaks of ranavirosis, their presence reduced the probability of experiencing highly virulent outcomes (North et al., 2015).

6. Community impacts of CMTV-like viruses in European amphibians

The narrow host range exhibited by British FV3-like ranaviruses stands in sharp contrast to the recent observations of lethal ranavirosis across western and central continental Europe. In the last decade, cases affecting amphibians reported in six continental European countries were consistently associated with another *Ranavirus* lineage, the CMTV-like ranaviruses. In the most extreme cases, six anuran and caudate amphibian species - from six genera and representing the complete amphibian community assemblage - have experienced simultaneous mortality events affecting larvae, juveniles and adults that caused sudden, multispecies and persistent population declines (Price et al., 2014). Although not all recent emergences of severe CMTV-like ranavirosis on the continent have so comprehensively affected host communities (Fig. 2), collectively they represent an enormously broad amphibian host range and are consistently causing mass mortality affecting multiple life history stages (Price et al., 2014; Miaud et al., 2016; Rijks et al., 2016; Rosa et al., 2017). When host range is combined from reports of emergent and lethal CMTV-like ranaviruses on the European continent it encompasses 15 amphibian species in 10 genera, or nearly 20% of European amphibian biodiversity recognized by the IUCN, and a squamate reptile (Temple and Cox, 2009; Price et al., 2014; Miaud et al., 2016; Rijks et al., 2016; Rosa et al., 2017), which serves to reinforce the emerging view of CMTV-like viruses as important pathogens with extremely broad host ranges.

There is a strong likelihood that additional mortality events caused by CMTV-like ranaviruses are going unreported. Mortality of Danish *Pelophylax esculentus* involving hundreds of frogs likely only came to the attention of the academic community because of a targeted appeal through the media and local environmental organisations (Ariel et al., 2009). Amphibians from Italy and Switzerland, countries that have yet to report ranavirosis caused by CMTV-like variants affecting wild amphibians, collected for research objectives unrelated to infectious diseases developed severe ranavirosis and experienced mass-mortality in captivity (Holopainen et al., 2009; Stohr et al., 2013). Captive conditions may have affected the ability to tolerate infection in these cases but it is perhaps more likely that these mortality events in captivity are symptomatic of more widespread but as yet unobserved epidemic disease outbreaks in the wild due to CMTV-like viruses. Underreporting aside, the preponderance of evidence indicates that CMTV-like variants are invasive in Continental European amphibian communities.

Incidents of mass die-offs in Spain, France, Portugal and the Netherlands are all recent, and were previously unreported by amphibian monitoring programmes active before the die-offs occurred and that detected the initial outbreaks. Phylogenetic analyses of CMTV-like ranaviruses responsible for these events consistently yield shallow clades exhibiting little or no variation among isolates derived from mixed host species assemblages and multiple sites within the respective regions (Price et al., 2014; Rijks et al., 2016; Rosa et al., 2017). These patterns are consistent with recent introduction events involving viruses with broad host range at time of introduction. The increased frequency of genes with evidence of positive selection in the genome of the type CMTV from Spain relative to other isolates of *Ranavirus* could also be interpreted as consistent with this model of invasion following recent introduction (Price, 2016). FV3-like ranaviruses are circulating on the Continent in both amphibians and reptiles and, in at least two systems, exhibit overlap with the distribution of CMTV-like ranaviruses, but are relatively rare (Price et al., 2014; Stöhr et al., 2015; Rosa et al., 2017). Assuming recent introduction of and invasion by CMTV-like variants, FV3-like ranaviruses may be undergoing replacement by invasive genotypes though recent studies suggest that overlapping range could result in 'mosaic' viruses generated by widespread recombination of two divergent genotypes (Price, 2016; Price et al., 2014; Rosa et al., 2017; Claytor et al., 2017). Given the phylogenetic relationship between CMTV-like variants affecting European herpetofauna and PPIV (Fig. 1), the recent emergence of CMTVs in European herpetofauna and the more historical evidence of asymptomatic infections in European freshwater fish species in the absence of observations of amphibian disease, it is possible that fish play an important role in the distribution of CMTV-like viruses affecting amphibians and reptiles in Europe.

7. Future directions

The list of locations and hosts from where ranavirus epidemics have been observed is growing but evidence of the age of the host-pathogen association is usually lacking. An exception is the Ambystoma-ATV system, where two lines of evidence support a very old association (Storfer et al., 2007; Epstein and Storfer, 2016). In long established systems, where host populations have engaged in a prolonged arms race with Ranavirus, examining host genomes for signatures of positive selection is likely to elucidate host defences which may in turn shed light on immune evasion and virulence evolution in the virus. In contrast, systems comprising asymptomatic infections occurring at low prevalence are somewhat counterintuitive to expectations of an evolutionary arms race (i.e. even where substitution rates are low, generation times of viruses are so short that they are expected to easily outpace their host), and may be explained by poor detection success and in other cases by a failure to identify the primary host. Ranaviruses are commonly held up as multi-host pathogens but transmission routes are generally poorly characterised in the wild and there have been few, if any, attempts to assess the relative quality of individual host species or quantify the role each plays in maintaining epidemics in the wild. We have outlined some basic geographic and host range patterns that suggest that FV3-like ranaviruses are predominantly amphibian generalists, ATV-like variants are fish specialists that in one case have host-switched to caudate amphibians, while CMTV-like ranaviruses affecting European herpetofauna may have the capacity to more routinely exploit the full breadth of the aquatic vertebrate community (Fig. 1). However, we postulate these relationships from an extremely limited evidence base and many questions remain as to how and when such systems can be maintained.

Even where active management and monitoring of populations precede observations of epidemic disease and patterns of viral diversity are suggestive of recent incursion, there is usually no direct and concrete evidence that ranaviruses were not circulating asymptomatically in the affected hosts or some reservoir species prior to the onset of disease (Price et al., 2014; Rosa et al., 2017). Surveys of archived specimens might be useful in this respect but the weight of indirect evidence in some cases already points strongly to recent human

behaviour creating opportunities for host and geographic range expansion (Price et al., 2016). It's possible that this has enabled ranavirus to escape its host-pathogen coevolutionary history and left naïve populations woefully exposed to a novel or re-emerging pathogen. After all, human factors have affected the direction of ranavirus evolutionary trajectories in other ways: ranavirus contamination of aquaculture and trade appears to have presented suitable opportunities for the evolution of increased virulence as well as recombination of divergent virus types (Storfer et al., 2007; Hoverman et al., 2011; Price, 2016; Claytor et al., 2017). However, there are striking differences in, for example, the exploitation of the assumed naïve communities following incursions into Europe by FV3-like and CMTV-like viruses and it is clear that virus genotype – likely modulated through an interaction with host and environmental factors - must have played a significant role.

A paucity of data has previously limited comparative genomic studies of *Ranavirus* but the recent focus on these pathogens as important threats to diverse hosts has resulted in the application of next-generation sequencing technologies to resolve phylogenetic relationships, understand evolutionary processes, and reveal the contributions of both to the epidemiology of this group. Genome content (and perhaps also the means to manipulate it) varies among even closely related ranaviruses and this is likely to impact on key viral traits such as infectivity, virulence and host range (Price, 2016). In addition to pairwise variation, genome content also varies systematically with genome size moving in both directions. There remains uncertainty about relationships among viruses at the root of the amphibian-associated ranaviruses (Jancovich et al., 2015; Stöhr et al., 2015; Price, 2016) but it appears there has been two independent cases of genome reduction in the amphibian-associated ranaviruses; one on the terminal branch to ATV, and the other on the ancestral branch leading to the last common ancestor of FV3-like and CMTV-like ranaviruses. Genomes of the remaining ATV-like ranaviruses are approximately 7–20 kb larger than ATV itself and the other amphibian-associated ranaviruses. It is possible that this high-level genome reorganisation is mediated through the purging of duplicated genes or pseudogenes and may be associated with a shift in host range to include amphibians. Genome size can increase through the acquisition of new genes and each of the three *Ranavirus* groups we've focused on contains lineage-specific genes (Price, 2016). Ranaviruses acquire new genes through lateral gene transfer from micro-organisms and their hosts as well as gene duplication events (Filee, 2009). Perhaps unsurprisingly, some of these newly acquired genes have undergone recent positive selection, which might be associated with adaptation to new hosts following host jumps (Abrams et al., 2013). The genome of CMTV, isolated during recurrent epidemics in Spain that have resulted in collapse of amphibian communities, has also been subject to widespread positive selection (Price, 2016), further highlighting how scans for adaptive change might identify both virulence genes and lineages undergoing geographic and/or host range expansion.

Although functional annotation of ranavirus genomes is generally lacking (homology searches yield no functional information for approximately two-thirds of ranavirus open-reading frames), gene knockout methodologies continue to offer a promising approach to explore how variable loci contribute to key viral traits (Robert and Jancovich, 2016). It is also possible that detection of more subtle changes to ranavirus genomes, such as silent shifts in GC content at the third codon position (synonymous substitutions) could yield information about use of hosts or environments. Finally, experimental challenges of amphibians and reptiles with members of the *Iridoviridae* only previously associated with infection of invertebrates can also elicit infections (Weinmann et al., 2007; Marschang et al., 2016) which suggests that broadening the scope of comparative genomic studies to include viruses of other genera in the family (such as the invertebrate viruses as well as those genera that are assumed to infect fish only) may help identify genes and mechanisms of genome evolution which determine virulence and host range.

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