Genetic differences in host infectivity affect disease spread and survival in epidemics

O. Anacleto[1], S. Cabaleiro[2], B. Villanueva[3], M. Saura[3], R.D. Houston[1], J.A. Woolliams[1], A. Doeschl-Wilson[1]
[1]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Division of Genetics and Genomics, Midlothian EH25 9RG, UK
osvaldo.anacleto@roslin.ed.ac.uk (Corresponding Author)
[2]INIA, Departamento de Mejora Genética Animal, Crta. La Coruña Km 7.5, 28040 Madrid, Spain
[3]Cluster de la Acuicultura de Galicia (CETGA), Spain

Summary

Genetic analyses of infectious disease data usually focus on host resistance to becoming infected or host ability to survive when exposed to infection. Increasing evidence however shows that risk and severity of disease outbreaks also depend on infectivity, which is the host ability to transmit infections. These traits may be under genetic control and correlated, but empirical evidence for this is currently sparse as appropriate data are lacking. This hampers introduction of effective breeding techniques that reduce disease spread. We developed a large-scale transmission experiment using 1800 fish, which provided suitable epidemic data for genetic analyses of infectivity, resistance to becoming infected and also tolerance to infection (ability to survive despite being infected). Scuticociliatosis, caused by the parasite *Philasterides dicentrarchi*, was used as a disease model. Since genetic studies of infectivity require natural transmission of the parasite, unrelated families of full-sibling fish (shedders) were artificially infected and distributed into 72 tanks, which in turn contained non-infected naïve fish (recipients). Disease outcome was observed by daily recording of infected and dead fish. Differences in Kaplan-Meier curves for recipient time to infection pooled by shedder or recipient families strongly suggested genetic variation in shedder fish infectivity, as well as in resistance, whereas evidence for genetic variance in tolerance was weak. Bayesian generalized linear mixed models fitted to daily counts of recipient fish with visual signs revealed that fish were on average 2.4 times more likely to become infected when exposed to the most infective shedder family compared to the least infective family. These results provide the first evidence for genetic variation in infectivity and offer new opportunities for implementing novel disease traits into animal breeding strategies to reduce infectious disease spread in livestock population.

Keywords: animal breeding, disease resistance, host infectivity, aquaculture

Introduction

Despite the compelling evidence for the effects of individual heterogeneity on the severity of disease epidemics, linking host genetic and epidemiological sources of variation has been a long-standing challenge in quantitative genetic studies of infectious disease. In particular, field studies often focus on host ability to survive when exposed to infection (Bishop et al. 2010). Whether an individual survives exposure to infection depend on the propensity of becoming infected (susceptibility) as well as its ability to survive, once infected

(tolerance). Moreover, the occurrence of superspreading events, in which a small proportion of highly infectious individuals are responsible the majority of transmission, has led to rapidly increasing interest in the genetic regulation of infectivity.

Infectivity can be defined as the ability of an infected host to transmit the infection to an average susceptible individual upon unit contact. Since this trait impacts the infection status of individuals in the same contact group, an individual's risk of infection is determined by its own susceptibility and the infectivity of its infected contacts. Genetic studies of infectivity come with specific data demands (for detailed description see Doeschl-Wilson *et al*. 2018). Until now, these data requirements and the lack of analytical tools have hampered simultaneous genetic analyses of susceptibility, tolerance and infectivity traits. In particular, it is not known to date whether host infectivity is under genetic control.

Here we present a transmission experiment in fish designed to generate epidemic data which allows quantitative genetic studies of susceptibility, tolerance and infectivity. By analysing family differences in these traits, we demonstrate, for the first time, that there is genetic variance in infectivity, in addition to susceptibility and tolerance, and how these traits contribute to survival.

Material and methods

The scuticociliatosis transmission experiment in Turbot

The experiment was conducted in CETGA in two consecutive trials, comprising in total 1800 offspring from 44 full-sibling families. As illustrated in Figure 1, to trigger the epidemics in the experiment, shedder fish were inoculated with a virulent strain of *P. dicentrarchi,* that causes scuticociliatosis and is one of the most important parasitological problems in marine aquaculture worldwide. These shedder fish were then introduced into 72 isolated tanks housing non-infected recipient fish, with 36 tanks per trial and no between tank transmission allowed. Each tank comprised 25 fish, with 5 fish from the same shedder family seeding the infection to 20 fish from 4 different recipient families (5 fish per recipient family). Also, each shedder family seeded the infection in 9 different tanks (9 experimental replicates per shedder family). To control for confounding between shedder fish infectivity and genetic susceptibility and infectivity of recipient fish, the same recipient family compositions were used across the 4 shedder families. This resulted in 9 different family compositions per trial, with each recipient family represented in 8 tanks and in 2 different family compositions.

The trials were terminated when no new infections were observed in most of the tanks, which was the case after 104 and 160 days in trials 1 and 2, respectively. Visual infection signs included exopthalmia, colour change or depigmentation, visible lesions or abnormal swimming behaviour. All fish that took part in the experiment were weighted and tissues were collected for parasite detection.

Statistical analyses

To compare infection and survival profiles associated with fish families, we used Kaplan-Meier estimators using time to signs (with censoring given by death by infection before appearance of visual signs), time to death and also for time from signs to death for fish that displayed visual infection signs. Daily counts of recipient fish with visual infection signs in each tank were used to estimate the effect of shedder fish family on time to infection (visual signs were assumed to be a proxy for time of infection). For a tank i and day t_j of the

experiment, the number of fish with visual signs, represented by $C_i(t_j)$ was assumed to follow a binomial distribution with parameters given by the number of susceptible individuals at day t_j in tank i, represented by $S_i(t_j)$, and p_j, which is the probability of a fish showing visual signs at t_j given it was susceptible prior to that day. This conditional probability is, by definition, the hazard of infection at day t_j (Rabe-Hesketh and Skrondal 2008) and can be modelled using a Bayesian generalised linear mixed model (GLMM) with a complementary log-log link function given by

$$\log(-\log(1-p_j)) = \alpha_j + b_i SF_i + RFC_i + I_{ij}$$

where α_j is the day-specific intercept representing a baseline hazard, SF_i is the shedder family at tank i and I_{ij} is an offset representing the proportion of infected fish at tank i and day t_j. Also, RFC_i is a random effect representing recipient family composition at tank i such that its covariance matrix incorporates family relationships within and between the compositions. A similar model was considered to estimate the shedder family effect on time to death by infection. Model parameters were estimated using the Rstan package in R.

Results

Figure 2 shows boxplots representing distributions of time to signs of infection and time between these signs and death for recipient fish from the two trials of the experiment. These plots indicate that it took longer for the recipients to become infected than to die following infection, with large variation in time to signs within and across trials. The large variation between trial 1 and 2 might be caused by a less virulent pathogen strain used in trial 2. Fish from both trials died quickly and with low variation following onset of signs (Figure 2). We found significant differences across the 36 recipient fish families in time to signs (log-rank test: $P<0.001$ for both trials) but small recipient family variation in time from signs to death (log-rank test: $P=0.053$ and $P=0.084$ for trial 1 and 2 respectively, also see Figure 3). These results may indicate large genetic variation in susceptibility but small variation in tolerance to the disease.

Shedder fish family had a strong effect on recipient time to signs, but had little influence on time from these signs to death (Figure 4). While recipients exposed to the most infective shedder families (C and F for trials 1 and 2 respectively, see Figures 4a and 4b) got infected at approximately twice the infection rate of recipients exposed to the least infective shedder families (B and G for trials 1 and 2 respectively, see Figures 4a and 4b; hazard ratio estimates: trial 1 posterior mean: 1.80, 95% CI: 1.37, 2.23; trial 2 posterior mean: 2.10, 95% CI: 1.60, 2.75), shedder family infectivity had a weak effect on the risk of death by infection (hazard ratio estimates: trial 1 posterior mean: 1.27, 95% CI: : 0.88, 1.84; trial 2 posterior mean: 0.98, 95% CI: 0.76, 1.21).

To evaluate the effect of the genetic composition of the tank members (accounting for combined differences in recipient susceptibility, infectivity and tolerance) on infection and survival time, random effects representing recipient family composition (RFC) were considered in all models. Variance components for RFC are much larger in the models for time to onset of visual signs when compared to estimates of the same component in models for time between infection signs and death (Table 1), suggesting that genetic composition of tank members plays a strong role in disease spread but has little influence on survival post infection.

Conclusion

Host genetics has yet not been fully exploited to reduce the impact of infectious disease in animal populations (Doeschl-Wilson *et al*. 2018). It is therefore crucial to understand and disentangle the effects of genetic diversity in different mechanisms of host defence to disease. With the temporal data generated from a transmission experiment using fish of defined family structure, we dissected the different sources of genetic variation on disease prevalence and subsequent mortality. Our study provided the first direct evidence that there are genetic differences in host infectivity and that the genetic make-up of a host can largely affect the survival of its group mates by affecting their risk of infection. These findings reveal new opportunities for devising more effective genetic disease control strategies by exploiting the genetic architecture underlying host susceptibility, infectivity and tolerance to disease (see, for example, Tsairidou *et al.* 2018).

The family structure of the fish considered in our transmission experiment is also expected to provide estimation of individual genetic risks for susceptibility, tolerance and infectivity (Saura *et al.* 2018). In particular, under the assumption of infectivity being a heritable trait, it can be defined as an indirect genetic effect (IGE, Griffing 1967). IGE models have recently been extended to incorporate the dynamics of infection processes (Anacleto *et al. 2015)* and are currently being applied to our experimental data to estimate genetic risks as well as heritabilities and environmental effects for both fish infectivity and susceptibility to scuticociliatosis.

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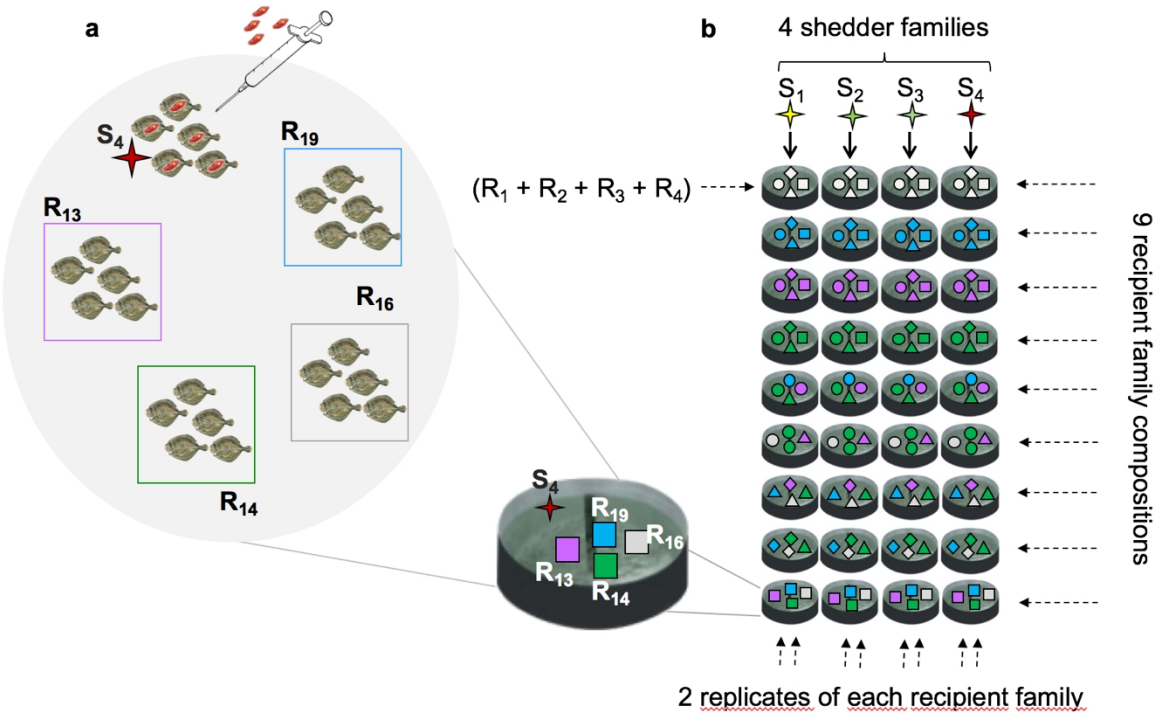

Figure 1: a Tank composition in our transmission experiment. Each of the 72 tanks comprised 5 artificially inoculated (shedder) fish from a single family and 20 susceptible (recipient) fish from 4 families. **b** Transmission experimental design in one of the trials. Each grey circle corresponds to one tank and a unique symbol-colour combination is assigned to each recipient (Ri) or shedder (Si) family in 36 tanks in one of the 2 trials of our experiment. The 4 shedder families (S_1 to S_4) were housed in 9 tanks each. Recipient families (18 per trial) were housed in tanks such that 9 recipient family combinations were created, which in turn were housed with each of 4 shedder families. Each recipient family was allocated in two recipient family compositions.

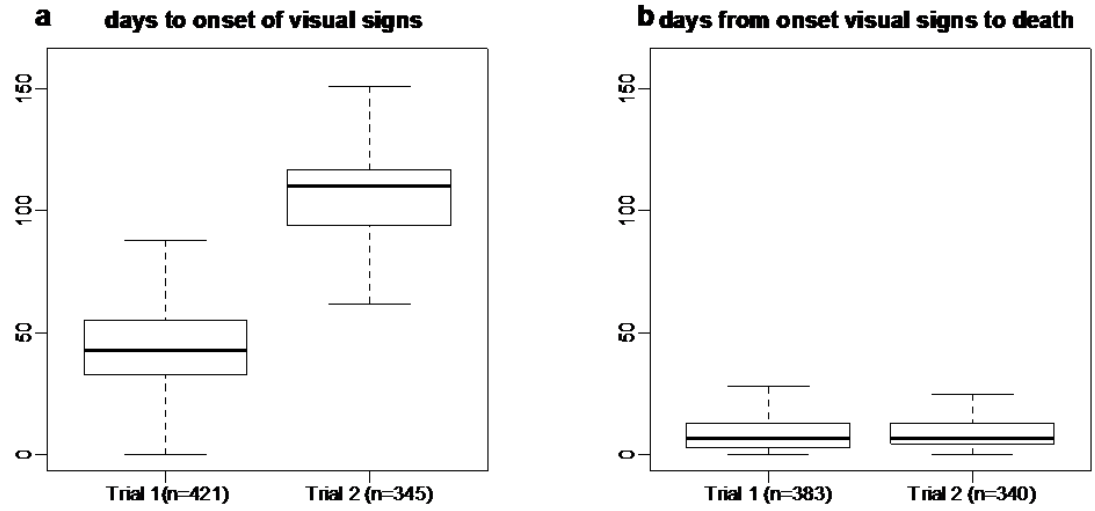

Figure 2: a Box plots of time to infection signs for recipient fish that displayed these signs in the two trials of our experiment. **b** Box plots of time from signs to death for recipient fish considered in **(a)** that died before the end of the experiment.

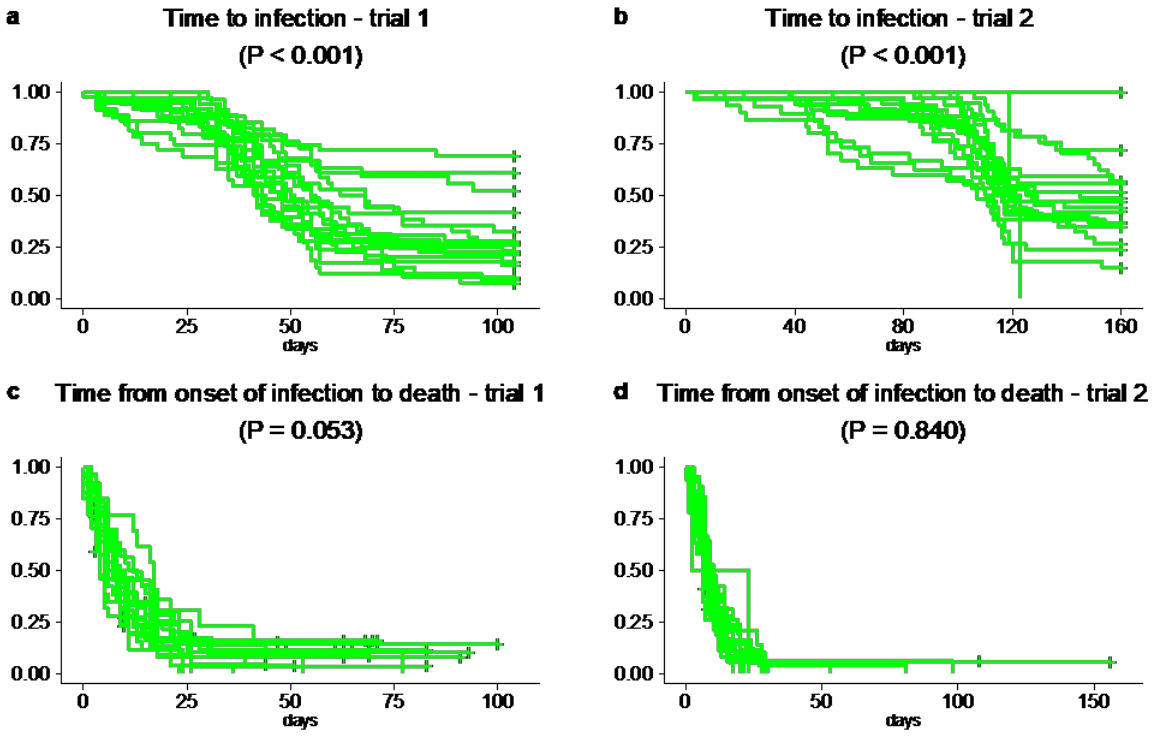

Figure 3. Evidence for genetic variation in susceptibility and tolerance to infection. Evolution of infection by Philasterides *dicentrarchi* **(a-b)** and survival post infection **(c-d)** in all families of recipient fish in trials 1 and 2 of the transmission experiment. The curves were obtained through family-based Kaplan-Meier plots for time to signs **(a-b)** and time from signs to death **(c-d).** P-values were calculated using the log-rank test for detecting family differences in Kaplan-Meier estimates.

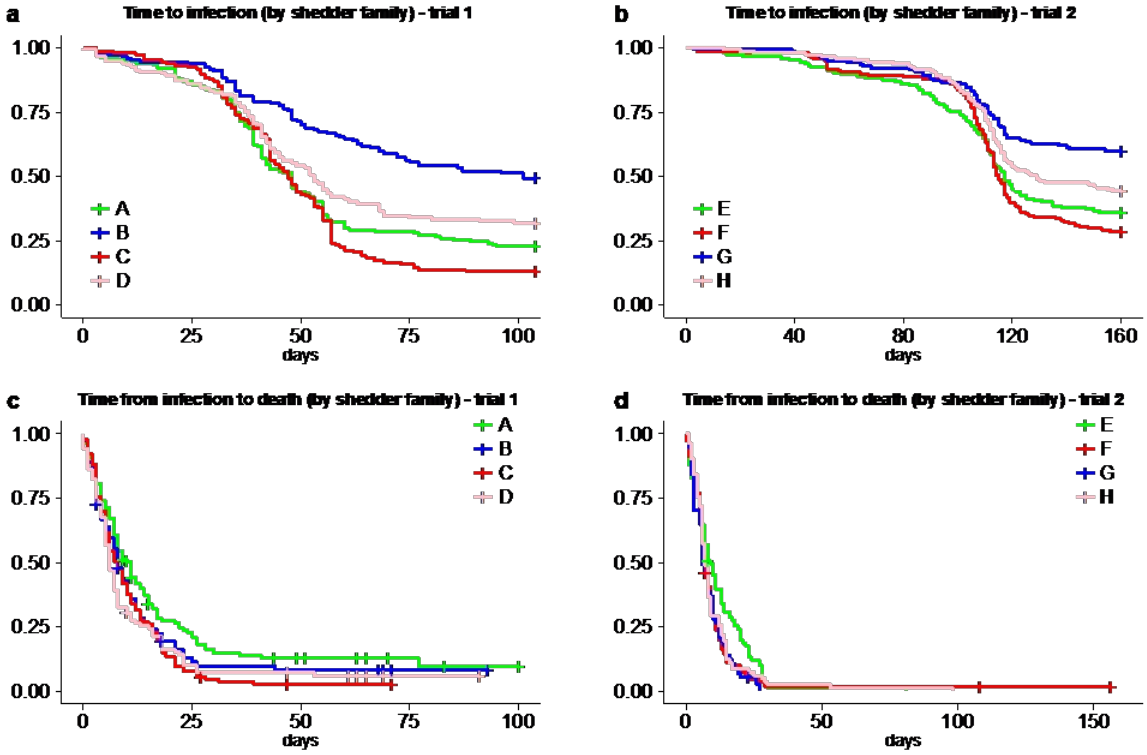

Figure 4. Evidence for genetic variation in host infectivity (ability to transmit infection). Evolution of the epidemics and survival post infection in recipient fish pooled by families of shedder fish shared across recipients. **(a)** and **(b)** Kaplan-Meier curves for time to signs of recipient fish from trials 1 and 2 by shedder family. **(c)** and **(d)** Kaplan-Meier curves for time from signs to death for recipient fish by shedder family, also for both trials of the experiment.

Table 1: Estimates of variance component representing recipient family composition effects, included in the generalised linear mixed models for time to signs and time from signs to death.

	time to infection				death by infection			
	mean	median	% credible interval		mean	median	% credible interval	
trial 1	0.64	0.47	0.18	1.85	0.12	0.07	0.00	0.53
trial 2	0.09	0.05	0.02	0.34	0.02	0.02	0.01	0.06