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Host population structure for tolerance determines the evolution of plant–virus interactions

Nuria Montes[1,2], Viji Vijayan[3] and Israel Pagán[3]

[1]Fisiología Vegetal, Departamento Ciencias Farmacéuticas y de la Salud, Facultad de Farmacia, Universidad San Pablo-CEU Universities, Boadilla del Monte (Madrid) 28668, Spain; [2]Servicio de Reumatología, Hospital Universitario de la Princesa, Instituto de Investigación Sanitaria (IIS-IP), Madrid 28008, Spain; [3]Centro de Biotecnología y Genómica de Plantas UPM-INIA and ETS Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid, Madrid 28223, Spain

Author for correspondence:
Israel Pagán
Email: jesusisrael.pagan@upm.es

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Summary

- Heterogeneity for plant defences determines both the capacity of host populations to buffer the effect of infection and the pathogen´s fitness. However, little information is known on how host population structure for tolerance, a major plant defence, impacts the evolution of plant–pathogen interactions.
- By performing 10 serial passages of *Turnip mosaic virus* (TuMV) in *Arabidopsis thaliana* populations with varying proportion of tolerant genotypes simulating different structures for this trait, we analysed how host heterogeneity for this defence shapes the evolution of both virus multiplication, the effect of infection on plant fecundity and mortality, and plant tolerance and resistance.
- Results indicated that a higher proportion of tolerant genotypes in the host population promotes virus multiplication and reduces the effect of infection on plant mortality, but not on plant fecundity. These changes resulted in more effective plant tolerance to virus infection. Conversely, a lower proportion of tolerant genotypes reduced virus multiplication, boosting plant resistance.
- Our work for the first time provides evidence of the main role of host population structure for tolerance on pathogen evolution and on the subsequent feedback loops on plant defences.

Introduction

Plant populations generally consist of individuals that differ in the level of defences against pathogens (Haldane, 1949; Agrawal & Lively, 2002; Sacristán & García-Arenal, 2008). The likelihood of an encounter with each of these defence phenotypes determines pathogen transmission and virulence (Elton, 1958; Lively, 2010; King & Lively, 2012); virulence is defined as the detrimental effect of pathogen infection on plant fitness (Read, 1994), measured as reduced fecundity and/or increased mortality (Day, 2002). Therefore, plant population structure for defence traits (i.e. the frequency of defence phenotypes) is regarded as a major determinant for pathogen evolution and emergence, and of plant population dynamics (Ostfeld & Keesing, 2012; Pagán *et al.*, 2016; Ekroth *et al.*, 2019). This is particularly true for plant viruses, which account for the largest fraction of plant emerging diseases (Anderson *et al.*, 2004).

Most studies on the relationship between population structure for host defences and pathogen epidemiology and evolution have focused on the effect of host variability for resistance/susceptibility (Ekroth *et al.*, 2019; González *et al.*, 2019). However, resistance (i.e. the host's ability to limit pathogen multiplication, Cooper & Jones, 1983), is not the only plant defence mechanism. Increasing evidence indicates that tolerance is a widespread and successful plant defence strategy against pathogens (Kutzer & Armitage, 2016; Pagán & García-Arenal, 2018, 2020). In plant–virus interactions, the term tolerance has been used to have different meanings. In their seminal work, Cooper & Jones (1983) defined tolerant hosts, as opposed to sensitive ones, as those 'that a specific virus can infect and in which it can replicate and invade without causing severe symptoms or greatly diminishing the rate or amount of plant growth or marketable yield (or fitness)'. Later work pointed to two limitations of this definition: (1) it made it difficult to determine if mild symptoms or no yield/fitness reduction were due to plant mechanisms to cope with the effect of infection (tolerance) or to reduced virus multiplication (resistance), and (2) it defined tolerance as an absolute term, when in nature different degrees of tolerance may occur (Boss & Parlevtiet, 1995). These authors proposed that tolerance should be viewed quantitatively as increased yield/fitness in relation to a given virus content, a definition adopted by part of the plant virology community (Jeger *et al.*, 2006; Alexander *et al.*, 2017), and in line with published literature on host–pathogen interactions at large (Little *et al.*, 2010; Råberg, 2014). These two views of tolerance currently coexist, and it is important to highlight that they are not

mutually exclusive: the Cooper & Jones (1983) definition would be an extreme case of that of Boss & Parlevtiet (1995) in which infection induces no yield/fitness loss at any virus load. Therefore, in this work we will refer to tolerance as a quantitative trait measuring the effect of infection on plant mortality and fitness corrected by virus load (Boss & Parlevtiet, 1995; Little *et al.*, 2010) and we will term the Cooper & Jones (1983) definition as 'absolute tolerance'.

Resistance and tolerance represent two different ways to cope with viruses (and pathogens in general): the former reduces both virus within-host multiplication and between-host transmission minimising virus fitness, whereas the later generally has the opposite effect (van den Bosch *et al.*, 2006). Because of this effect of amplification on infection risk, tolerance is often discouraged as a disease control strategy in crops, although it can be of agronomic interest in certain circumstances (for instance, if large reservoirs of virus exist under conditions from which they cannot be eradicated) (Hull, 2014). However, in wild plant populations, in which individuals constantly compete for resources, tolerance can be a beneficial trait as it increases infection pressure over nontolerant genotypes (Cronin *et al.*, 2014). Indeed, virus-mediated competition has been proposed to explain the replacement of native flora by invasive plant species (Malmstrom *et al.*, 2005). Consequently, resistance and tolerance may exert different selection pressures on the virus, which may have contrasting but equally important impacts on its evolution: resistance imposes selection for higher pathogen infectivity to overcome this host defence. Conversely, tolerance favours more exploitative pathogens (i.e. those achieving higher within-host multiplication) as they do not pay the cost of higher titres in terms of increasing host mortality (Roy & Kirchner, 2000; van den Bosch *et al.*, 2006). Which is the predominant plant defence strategy has also consequences for host population dynamics: virus evolution to overcome plant resistance will impose a strong selection on the plant to evolve new efficient resistance, whereas tolerance is thought to be a more durable defence strategy as it does not reduce virus fitness (Cronin *et al.*, 2014).

The relevance of tolerance as a host defence resulted in the development of a body of theory aimed at predicting its impact on pathogen evolution. Most of these works focused on tolerance to infection-induced reduction of host lifespan (mortality tolerance), as shorter lifespan has an effect on both the host and the pathogen fitness by reducing the chances for host progeny production and for pathogen transmission (Read, 1994; Best *et al.*, 2008). Mathematical models by Miller *et al.* (2005, 2006, 2007) predicted that higher tolerance in the host population will lead to lower pathogen virulence, with its multiplication depending on the shape of the virulence-tolerance function: if virulence decreases nonlinearly with tolerance, low levels of this defence mechanism will select for slower replicating pathogens and the opposite will occur at high tolerance levels. If virulence decreases monotonically with tolerance, selection will always result in increased pathogen multiplication. By contrast, it has been shown theoretically that selection for mortality tolerance might promote an increase in both pathogen virulence and multiplication when these changes increase the pathogen´s transmission (Restif & Koella, 2003; Best *et al.*, 2014; Vitale & Best, 2019). Despite differences in their predictions, models agree in pointing out that more exploitative pathogens would be highly virulent in nontolerant hosts, even if they have no effect in tolerant ones (Restif & Koella, 2003; Miller *et al.*, 2006). In addition to higher mortality, the effect of infection can be also manifested in a reduced host progeny. Fewer studies have modelled pathogen evolution in response to tolerance to such effects (i.e. fecundity tolerance), and not always with the same results than in response to mortality tolerance (Best *et al.*, 2008). For instance, Restif & Koella (2004) found a negative association between virulence and fecundity tolerance, whereas Best *et al.* (2010) predicted optimal fecundity tolerance at intermediate levels of virulence such that, depending on the virulence onset, this trait could be positively or negatively associated with tolerance. These authors also found that higher host fecundity tolerance increased pathogen multiplication. Therefore, mortality and fecundity tolerance may differentially influence pathogen evolutionary dynamics, with their effects depending on the shape of the tolerance-virulence function and/or on the virulence onset. Fecundity and mortality tolerance may also have different impacts on the plant population: the former allows maintaining host population sizes even at high pathogen prevalence, the later may not if higher mortality tolerance comes at the cost of reduced fitness.

All models discussed above stem from classical mathematical elaborations by Anderson and May on the drivers of host–pathogen coevolution developed to be applicable to both extracellular (bacteria, fungi) and intracellular (viruses) pathogens, as well as to plant and animal hosts (Anderson & May, 1982, 1983). Indeed, models specifically constructed for plant viruses have led to similar predictions. For instance, mathematical elaborations under the assumption of a negative association between tolerance and virulence, and considering the virus vector dynamics, also predicted that plant tolerance favours virus evolution towards higher multiplication levels (van den Bosch *et al.*, 2006, 2007). Zeilinger & Daugherty (2014) analysed this question in more detail, and their model found that higher virus multiplication was favoured only if tolerant plants were more attractive to vectors if not viruses evolving in nontolerant hosts would have a selective advantage. In the same line, models based on data from grass species infected by *Barley yellow dwarf virus* (BYDV), and accounting for plant developmental tempo, predicted that short-lived plants would be more tolerant than long-lived ones, the former supporting higher virus titres (Cronin *et al.*, 2014). Interestingly, these models also predicted that proximity to a tolerant plant population would increase virus pressure in a nontolerant one (Zeilinger & Daugherty, 2014; Jeger *et al.*, 2018).

Numerous experimental analyses have indicated that phenotypic structure for tolerance in animal and plant populations varies along a continuum from fixation (Roy *et al.*, 2000; Carr *et al.*, 2003; Lefèvre *et al.*, 2011) to no tolerance (Montes *et al.*, 2020), with a range of intermediate levels in between (Råberg *et al.*, 2007; Pagán *et al.*, 2008; Hayward *et al.*, 2014). It is thought that structure for tolerance can be maintained in the host population if tolerance has a cost in terms of host fitness or by reducing defences to other pathogens (Restif & Koella, 2003;

Fornoni *et al.*, 2004; Vitale & Best, 2019), and evidence of such costs have been reported in several plant–pathogen (including virus) interactions (Simms & Triplett, 1994; Koskella *et al.*, 2002; Montes *et al.*, 2020). These observations strongly suggest that pathogens commonly face heterogeneous plant population structures for tolerance; yet analyses of pathogen evolution in response such structure are scant and sometimes contradictory, particularly for plant viruses. For instance, wheat and maize cultivars tolerant to BYDV and *Maize streak virus*, respectively, have been used for decades, suggesting that in these cases viruses infecting tolerant plants maintained low aggressiveness (induced mild symptoms) and virulence (did not affect progeny production) even if they multiplied at high levels in the plant (Buddenhagen & Bosque-Perez, 1999; Hull, 2014; Walls *et al.*, 2019). This would agree with lower virulence and higher multiplication evolving in response to tolerance (van den Bosch *et al.*, 2006, 2007; Cronin *et al.*, 2014). At odds, deployment of zucchini cultivars with absolute tolerance to *Zucchini yellow mosaic virus* (ZYMV) resulted in the appearance of more aggressive and virulent (affecting fruit development) virus strains but did not alter virus multiplication levels (Desbiez *et al.*, 2003). Similarly, in a 2-yr field trial, BYDV infecting tolerant switchgrass genotypes showed higher virulence than when infecting nontolerant plants, with no differences in virus multiplication (Alexander *et al.*, 2017). Therefore, both virus evolution in response to plant population structure for tolerance, and the potential feedback loops on plant defences, are still poorly understood.

Here, we analysed this question using *Turnip mosaic virus* (TuMV, *Potyviridae*) and *Arabidopsis thaliana* (from here on 'Arabidopsis', Brassicaceae). TuMV is commonly found in wild populations of Arabidopsis, mostly in a single infection, at up to 60% prevalence (Pagán *et al.*, 2010), indicating that the Arabidopsis–TuMV interaction is significant in nature. TuMV infection affects Arabidopsis flower and silique viability, which may severely reduce plant fertility and often prevents reproduction (Sánchez *et al.*, 2015; Montes *et al.*, 2020). Also, virus infection greatly shortens plant lifespan (Vijayan *et al.*, 2017), and particularly the Arabidopsis reproductive period, thereby affecting progeny production (Montes *et al.*, 2020). Therefore, TuMV effects on both plant fecundity and mortality affect host fitness. Moreover, virus-induced plant mortality also determines the TuMV infectious period and therefore transmission, as this virus is aphid vectored (Walsh & Jenner, 2002). We have recently shown that Arabidopsis displays fecundity and mortality tolerance to TuMV, which vary quantitatively across Arabidopsis genotypes (but is never absolute) with medium–high heritability and are dependent on the plant genotype (Montes *et al.*, 2020). Long-lived genotypes with low seed production to total biomass ratio (Group 1 genotypes, Pagán *et al.*, 2008) are less tolerant to both virus-induced reductions of mortality and fecundity than short-lived genotypes that have a high seed to biomass ratio (Group 2 genotypes), which is in agreement with theoretical models (Miller *et al.*, 2007; Cronin *et al.*, 2014). The detailed characterisation of Arabidopsis tolerance to TuMV allowed the construction of host populations with different structures for tolerance (i.e. different proportions of genotypes with higher and lower tolerance), and to analyse changes in infection traits. Particularly, we studied the evolution of TuMV within-host multiplication and virulence, of host tolerance, and the relationships between these traits. We also explored whether the observed changes were associated with genotype-specific host adaptation and/or with the initial levels of TuMV virulence.

Materials and Methods

Virus isolates and Arabidopsis genotypes

UK1-TuMV (acc. no. AB194802) and JPN1-TuMV (acc. no. KM094174) were used. Viruses were derived from plasmids containing molecular clones of each isolate, both kindly provided by Professor Fernando Ponz (CBGP, Madrid, Spain) (Sánchez *et al.*, 1998; López-González *et al.*, 2017). Because of low plasmid infectivity, we first inoculated them in *Nicotiana benthamiana* plants for virus multiplication, and sap from these plants was used for Arabidopsis inoculation (see below). UK1-TuMV and JPN1-TuMV have different levels of virulence in Arabidopsis (Sánchez *et al.*, 2015; Montes *et al.*, 2020), which allowed exploring how host population structure for tolerance affected virus evolution according to the virulence onset. We used four Arabidopsis genotypes distributed between the two previously defined plant allometric groups (Pagán *et al.*, 2008), which differ in mortality and fecundity tolerance to TuMV (Montes *et al.*, 2020): Cum-0 (Cumbres Mayores, Spain) and Ll-0 (Llagostera, Spain), which belonged to Group 1 and were less tolerant (mortality and fecundity) to TuMV; and genotypes Col-0 (Columbia, Unknown) and L*er* (Landsberg, Poland), which belonged to Group 2 and were more tolerant to TuMV. Seeds were stratified for 7 d at 4°C in 15-cm-diameter pots, 0.43 l volume containing a 3 : 1, peat : vermiculite mix. Afterwards, pots were moved for seed germination and plant growth to a glasshouse at 22°C, 16 h light (intensity: 120–150 μmol s m^{-2}), with 65–70% relative humidity, as an approximation to conditions in which the Arabidopsis life cycle takes place in temperate climates (Pagán *et al.*, 2010; Manzano-Piedras *et al.*, 2014).

Serial passages

Ll-0 (less tolerant, LT) and Col-0 (more tolerant, MT) were used to construct host populations with 100% LT plants (P0, only Ll-0 individuals), 75% LT/25% MT (P25), 50% LT/50% MT (P50), 25% LT/75% MT (P75) and 100% MT plants (P100, only Col-0 individuals). Each population consisted of 120 plants: 60 were infected with JPN1-TuMV and 60 with UK1-TuMV. The 60 plants infected by each virus were divided into three groups of 20, maintaining the corresponding proportion of LT/MT plants, such that three lineages per virus and host population structure were created (Fig. 1a). Plants were mechanically inoculated with infected tissue from *N. benthamiana* plasmid-inoculated plants ground in 0.1 M Na_2HPO_4 + 0.5 M NaH_2PO_4 + 0.02% sodium diethyldithiocarbamate when plants were at developmental stages 1.05–1.06 (Boyes *et al.*, 2001). For each treatment, three leaves per plant were collected 25 d

postinoculation (dpi) and pooled according to host population and virus lineage. Half of the pooled plant material was processed to determine virus presence using RT-qPCR (see below), and the other half was used to mechanically inoculate another set of 20 plants conforming a new host population with the same structure for tolerance as that from which the inoculum was collected. This procedure was repeated until 10 passages of horizontal transmission were completed (Fig. 1a). The dpi for tissue collection were chosen such that viruses infecting less tolerant plants had lesser chances to initiate the next passage than those infecting more tolerant ones, reflecting the effect of tolerance on the likelihood of transmission by its effect on the infectious period.

Analysis of TuMV evolution after serial passages

Four plant genotypes were selected: Ll-0 and Col-0 were common to all host populations used in serial passages, and Cum-0 and L*er* allowed the exploration whether virus evolution, and its consequences for plant tolerance, after passages were associated with genotype-specific host adaptation or with a broader response to the level of tolerance in the host population. Sap extracts of UK1-TuMV and JPN1-TuMV infected plants of the tenth passage were used to inoculate seven plants per virus lineage from each of the four plant genotypes, seven plants per genotype were inoculated with the *N. benthamiana* leaf material used to inoculate the initial passage (ancestral viruses), and other seven plants per genotype were mock inoculated (Fig. 1b). Virus multiplication, effect of virus infection on seed production and on plant lifespan, and the level of host fecundity and mortality tolerance (see below) were determined.

Quantification of virus multiplication

Virus multiplication was quantified as virus RNA accumulation 25 d postinoculation using RT-qPCR. For each plant, four leaf discs of 4 mm in diameter from systemically infected rosette leaves were collected. Total RNA extracts were obtained using TRIzol® reagent (Life Technologies, Carlsbad, USA), and 20 ng of total RNA were added to the Brilliant III Ultra-Fast SYBR Green qRT-PCR Master Mix (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's recommendations. Specific primers were used to amplify a 70-nt fragment of the TuMV coat protein (CP) gene (Lunello *et al.*, 2007). Each sample was assayed by triplicate on a Light Cycler 480 II real-

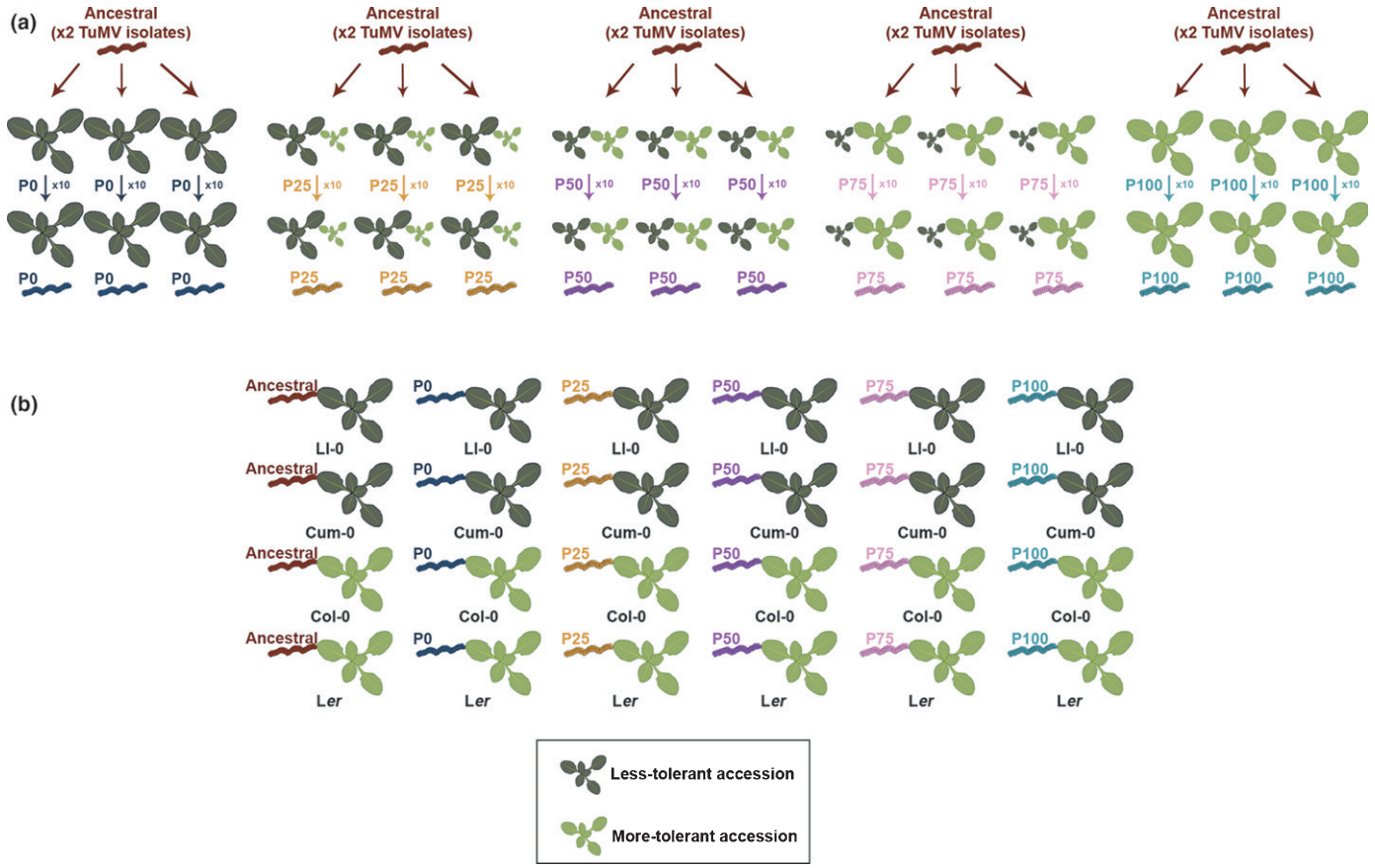

Fig. 1 Experimental design. (a) Ten serial passages of horizontal transmission of two TuMV isolates were performed in *Arabidopsis thaliana* populations with no tolerant (P0, black), 25% tolerant (P25, orange), 50% tolerant (P50, purple), 75% tolerant (P75, pink) and 100% tolerant (P100, blue) plants. Size of the plants indicates the corresponding proportion of more tolerant (light green) and less tolerant (dark green) genotypes. Three lineages per population structure for tolerance were generated. (b) Virus lineages obtained after serial passages plus the ancestral isolates were inoculated in plants of two more tolerant (Col-0 and L*er*) and two less tolerant (Cum-0 and Ll-0) genotypes.

time PCR system (Roche, Indianapolis, IN, USA). Absolute virus RNA accumulation was quantified as ng of virus RNA/μg of total RNA utilising internal standards, which consisted of a 10-fold dilution series of either UK1-TuMV or JPN1-TuMV RNA ranging from 2×10^{-3} ng to 2×10^{-7} ng.

Virulence and tolerance measures

We considered the effect of TuMV infection both on plant fecundity and on plant mortality. Plant fecundity was measured as per plant seed weight (*SW*). Seed viability, estimated as per cent germination, did not significantly differ between mock-inoculated (90.0–99.5%) and infected (92.3–99.1%) plants ($\chi^2 \leq 1.05$; $P \geq 0.488$). Also, virus infection did not affect the weight of a single seed (Wald's test $\chi^2 \leq 0.87$; $P \geq 0.132$). Therefore, *SW* similarly reflects the number of viable seeds in both mock-inoculated and infected plants. Because TuMV infection has sublethal effects, and to account for the effect of infection on the infectious period, plant mortality was measured through plant lifespan (*LP*), defined as the time from plant inoculation to senescence. We quantified virulence both as infection-induced reduction of plant fecundity (fecundity virulence) and lifespan (mortality virulence). Fecundity virulence was estimated as one minus the ratio of the total seed weight of infected (SW_i) to mock-inoculated (SW_m) plants, $1 - (SW_i/SW_m)$, and the same calculation was used for mortality virulence using *LP*. Following Little *et al.* (2010) and Zeilinger & Daugherty (2014), fecundity and mortality tolerances of each Arabidopsis genotype were calculated as the slope of the linear regression of *SW* and *LP*, respectively, to virus accumulation considering both infected and mock-inoculated plants.

Statistical analysis

First, we analysed the presence of outliers in the distribution of values of virus multiplication, *LP* and *SW* using Grubbs' test. As for the two tolerance measures only three values per treatment (one per lineage) were available, outliers were detected using a shifting z-score procedure developed for very small sample sizes (van Selst & Jolicoeur, 1994) (Supporting Information Dataset S1; Table S1). Virus accumulation and $1 - (SW_i/SW_m)$ were not normally distributed, and variances were heterogeneous according to Kolmogorov–Smirnov and Levene's tests, respectively. Therefore, they were fitted to a log-normal distribution; whereas $1 - (LP_i/LP_m)$, and fecundity and mortality tolerance were fitted to a normal distribution according to Akaike's Information Criteria (R package: RRISKDISTRIBUTIONS; Belgorodski *et al.*, 2017). Differences between viruses, plant genotypes and population structures were analysed by generalised linear mixed models (GzLMMs) considering virus isolate and population structure as fixed factors, and Arabidopsis genotype as a random factor in a full factorial model that included interactions between factors. This model was simplified for genotype-specific and virus-specific analyses by removing the corresponding factors and interactions. Statistical significance was analysed using Wald's chi-squared test (fixed factors) or likelihood ratio (random factor) tests. Analyses including allometric group, instead of host genotype, as factor were performed using generalised linear models (GzLMs) as all factors were considered as fixed. Variance components were determined using GzLMMs by the REML method (Lynch & Walsh, 1998). GzLMMs were performed using R-libraries LME4, NLME and LMERTEST (Bates *et al.*, 2015; Kuznetsova *et al.*, 2017; Pinheiro *et al.*, 2020). Analyses indicated no differences between lineages passaged in the same host population structure, and virus lineage was not considered as a factor. Relationships between traits were analysed using linear, quadratic, cubic, logarithmic, exponential and inverse regressions. Statistical analyses were conducted using R v.3.6.3 (R Core Team, 2020) (Notes S1).

Results

Full factorial GzLMMs indicated that all traits significantly differed according to the population structure in which the virus was passaged (Wald's test $\chi^2 \geq 16.51$, $P \leq 5 \times 10^{-3}$). Virus multiplication and mortality/fecundity virulence also varied depending on the virus isolate (Wald's test $\chi^2 \geq 16.45$, $P < 1 \times 10^{-4}$). The variance component attributable to host genotype varied between 1.2% (mortality tolerance) and 36.36% (fecundity tolerance). Importantly, in all traits either both pairwise interactions of population structure with the other two factors or the triple interaction were significant (Wald's test $\chi^2 \geq 9.53$, $P \leq 0.049$). Therefore, we analysed differences between viruses passaged in each population structure for each Arabidopsis genotype and virus isolate separately. Analyses described below follow this scheme. GzLMs using the allometric group (i.e. Col-0 and L*er* vs Cum-0 and Ll-0, which differ in both tolerances to TuMV) instead of genotype revealed no significant effect of this factor in any trait (Wald's test $\chi^2 \leq 7.52$, $P \geq 0.107$), and was not considered for further analyses.

Effect of Arabidopsis population structure on TuMV multiplication

UK1-TuMV multiplication varied between viruses passaged in the various host population structures (Wald's test $\chi^2 \geq 32.83$, $P < 1 \times 10^{-4}$). A general trend towards higher virus multiplication of viruses passaged in populations with larger proportions of MT plants (P75 and P100) was observed (Fig. 2). However, the pattern of variation in comparison with the ancestral virus depended on the plant genotype. In Ll-0 and Col-0, P0, P25 and P50 viruses showed similar values than the ancestral virus ($P \geq 0.067$); and all were significantly lower than P75 and P100 viruses ($P \leq 0.039$). In Cum-0 and L*er*, viruses passaged in P75 and P100 displayed similar accumulation as the ancestral virus ($P \geq 0.102$), with P0, P25 and P50 viruses showing lower loads ($P \leq 0.017$) (Fig. 2). This suggested that virus multiplication could have changed in response to both the structure of the host population and the specific plant genotypes conforming the populations. Therefore, it could be argued that the observed results were due to genotype-specific adaptation rather than to virus evolution in response to population structure for tolerance. To explore this possibility, we calculated the percentage of the

variance in virus RNA accumulation explained by host genotype and by population structure. This analysis indicated that the later factor explained about twice more variance than the former (19% and 35%, respectively). In addition, we divided host genotypes in two types: those that conformed the populations in which the viruses were passaged (Ll-0 and Col-0) and those that did not (Cum-0 and L*er*), and we constructed GzLMs incorporating this factor. Results indicated no significant differences in virus accumulation according to the host grouping (Wald's test $\chi^2_{1,417} = 2.18$, $P = 0.140$).

Differences between JPN1-TuMV viruses existed (Wald's test $\chi^2 \geq 26.32$, $P < 1 \times 10^{-4}$), but were less pronounced (Fig. 2). P0, P25 and P50 viruses infecting L*er* had lower accumulation ($P \leq 0.012$), and P75 and P100 viruses infecting Cum-0, Ll-0 and Col-0 accumulated more ($P \leq 0.049$), than the ancestral. No significant differences were observed in the rest of pairwise comparisons with the ancestral virus ($P \geq 0.187$) (Fig. 2). Again, population structure explained a higher proportion of the variance in virus multiplication than host genotype (38% vs 11%). In agreement, host grouping as above did not affect virus multiplication (Wald's test $\chi^2_{1,410} = 0.82$, $P = 0.438$), and explained no variance in this trait.

Therefore, TuMV generally evolved towards increased multiplication levels when passaged in populations with a higher frequency of more tolerant plants than in those conformed mostly by less tolerant ones. This effect was stronger in UK1-TuMV than in JPN1-TuMV viruses.

Effect of Arabidopsis population structure on the evolution of TuMV mortality virulence

Virulence modulates virus transmission through its effect on host mortality, which reduces the infectious period, an effect compensated by tolerance. Our serial passage experiment reflected this link as only viruses infecting plants that survived until tissue sampling were collected to initiate the next passage, which was more likely in tolerant plants. Accordingly, we started by quantifying mortality virulence as the effect of virus infection on plant lifespan ($1 - (LP_i/LP_m)$).

For UK1-TuMV, passaged viruses showed a general trend towards lower $1 - (LP_i/LP_m)$ than the ancestral (Wald's test $\chi^2 \geq 55.81$, $P < 1 \times 10^{-4}$). In all four genotypes, P0 viruses showed the largest reduction in virulence and P100 viruses the lowest ($P = 1 \times 10^{-4}$). P25, P50 and P75 viruses showed intermediate

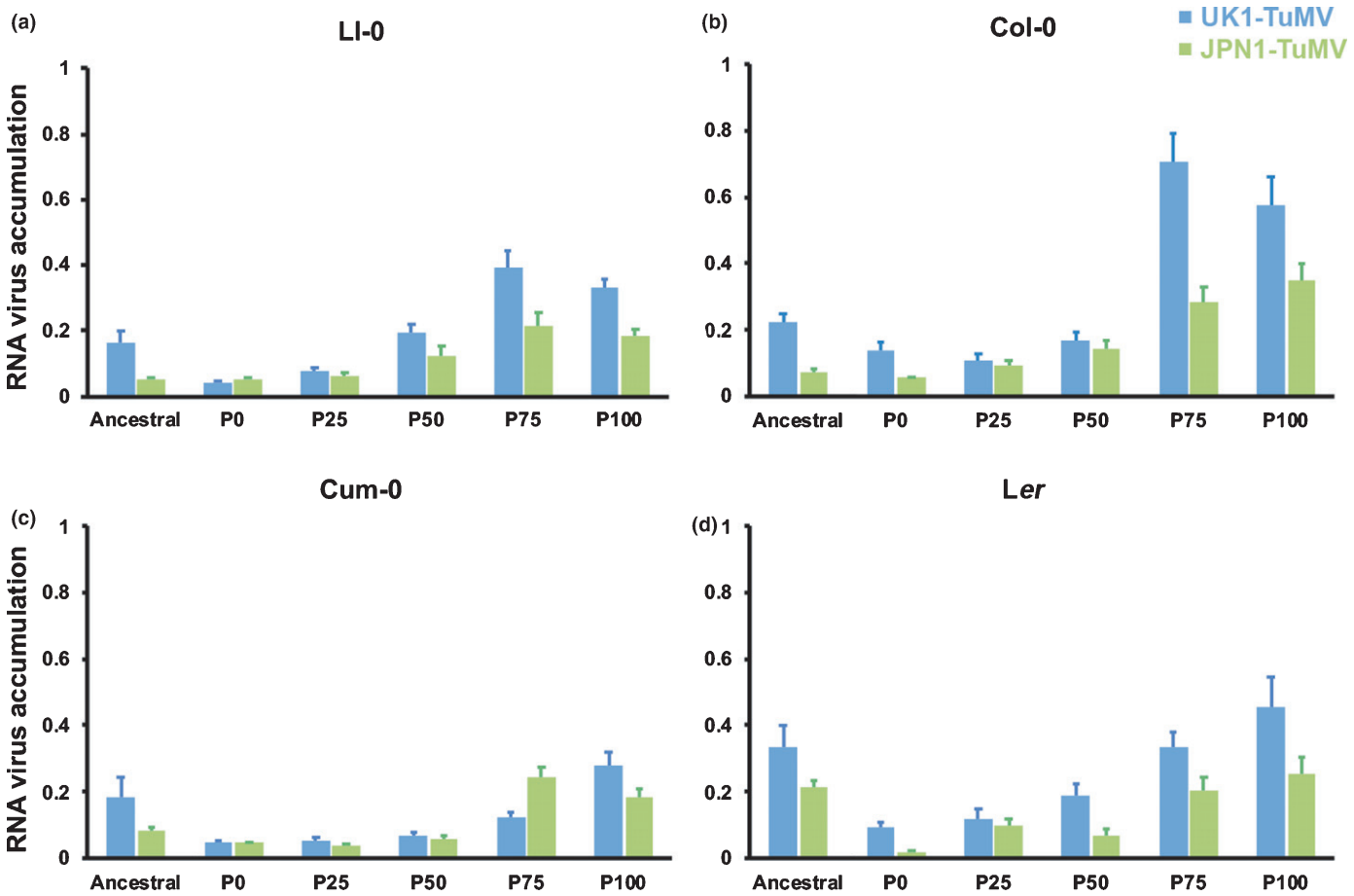

Fig. 2 TuMV accumulation in *Arabidopsis thaliana* genotypes. Accumulation of viral RNA (ng of virus RNA μg^{-1} of total RNA) in Ll-0 (a), Col-0 (b), Cum-0 (c) and L*er* (d). Data are given as mean ± SE of seven replicates per three lineages. TuMV isolates: UK1-TuMV (blue), JPN1-TuMV (green). Ancestral, P0, P25, P50, P75 and P100 refers to the host population from which the virus was obtained.

values (Fig. 3). Population structure explained a much larger proportion of the variance in $1-(LP_i/LP_m)$ than did the host genotype (45% vs 5%). Mortality virulence was similar in host genotypes that did or did not conform the plant populations in which viruses were passaged (Wald's test $\chi^2_{1,417}=0.73$, $P=0.392$), discarding a significant role of host genotype-specific adaptation in the observed patterns.

JPN1-TuMV also differed in morality virulence in all four genotypes (Wald's test $\chi^2 \geq 26.03$, $P<1\times10^{-4}$) (Fig. 3). However, differences between the ancestral and the passaged viruses were rarely significant: In Col-0 plants, no significant differences in $1-(LP_i/LP_m)$ between the ancestral and the passaged viruses were detected ($P\geq0.149$), and in Cum-0 and L*er* only P25 viruses had significantly higher values than the ancestral ($P\leq0.021$). By contrast, in Ll-0 plants all passaged viruses showed a significant reduction of $1-(LP_i/LP_m)$ compared with the ancestral ($P\leq2\times10^{-4}$). Differences in $1-(LP_i/LP_m)$ between passaged viruses were observed, but no general pattern was apparent (Fig. 3). In accordance, host population structure and genotypes explained a small proportion of the variance in morality virulence (16% and 4%, respectively).

Overall, serial passaging tended to reduce UK1-TuMV mortality virulence, with viruses from fully less tolerant populations always evolving towards lowest virulence and passages in fully more tolerant populations having the smallest effect on mortality virulence. For JPN1-TuMV viruses, host population structure had a limited effect on this trait.

Effect of Arabidopsis population structure on the evolution of TuMV fecundity virulence

Virus infection may also affect plant fitness. Therefore, we also analysed the evolution of fecundity virulence quantified as the effect of virus infection on seed weight ($1-(SW_i/SW_m)$) (Fig. 4).

In all genotypes, UK1-TuMV fecundity virulence varied between ancestral and passaged viruses (Wald's test $\chi^2\geq16.74$, $P\leq5\times10^{-3}$) (Fig. 4), mostly because of a significant reduction of $1-(SW_i/SW_m)$ in P0 viruses compared with the ancestral ($P<1\times10^{-4}$). No other passaged virus differed in $1-(SW_i/SW_m)$ from the ancestral except for P75 and P100 viruses infecting Ll-0 ($P\leq0.051$). Accordingly, host genotype and population structure explained a small percentage of the variance in fecundity virulence (5% and 2%, respectively). Also, viruses did not differ in $1-(SW_i/SW_m)$ between plant genotypes that did or did not conform host populations in which they were passaged (Wald's test $\chi^2_{1,417}=0.47$, $P=0.491$).

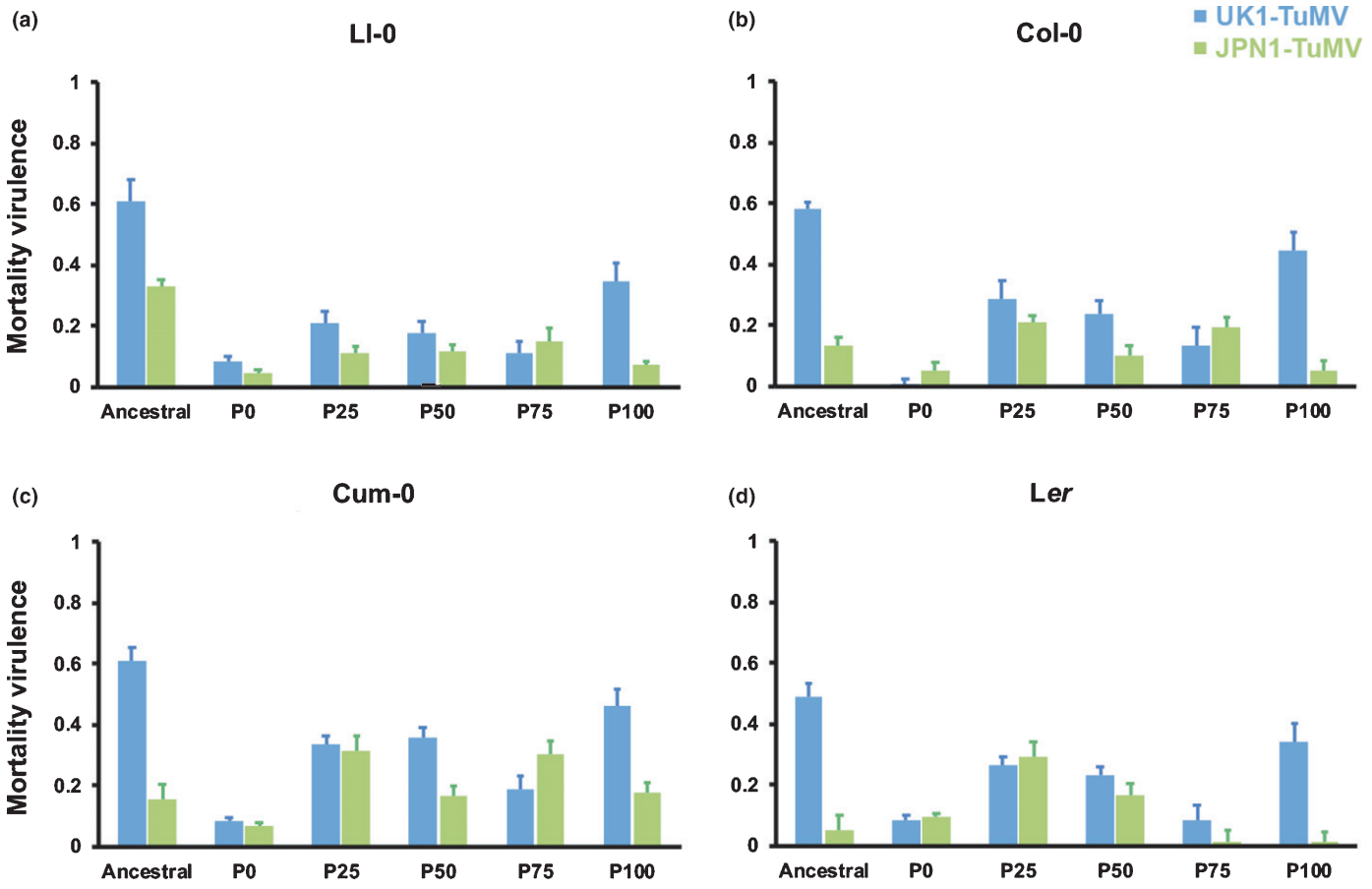

Fig. 3 TuMV mortality virulence in *Arabidopsis thaliana* genotypes. Effect of virus infection on plant lifespan ($1-(LP_i/LP_m)$) in Ll-0 (a), Col-0 (b), Cum-0 (c) and Ler (d). Data are given as mean ± SE of seven replicates per three lineages. TuMV isolates: UK1-TuMV (blue), JPN1-TuMV (green). Ancestral, P0, P25, P50, P75 and P100 refers to the host population from which the virus was obtained.

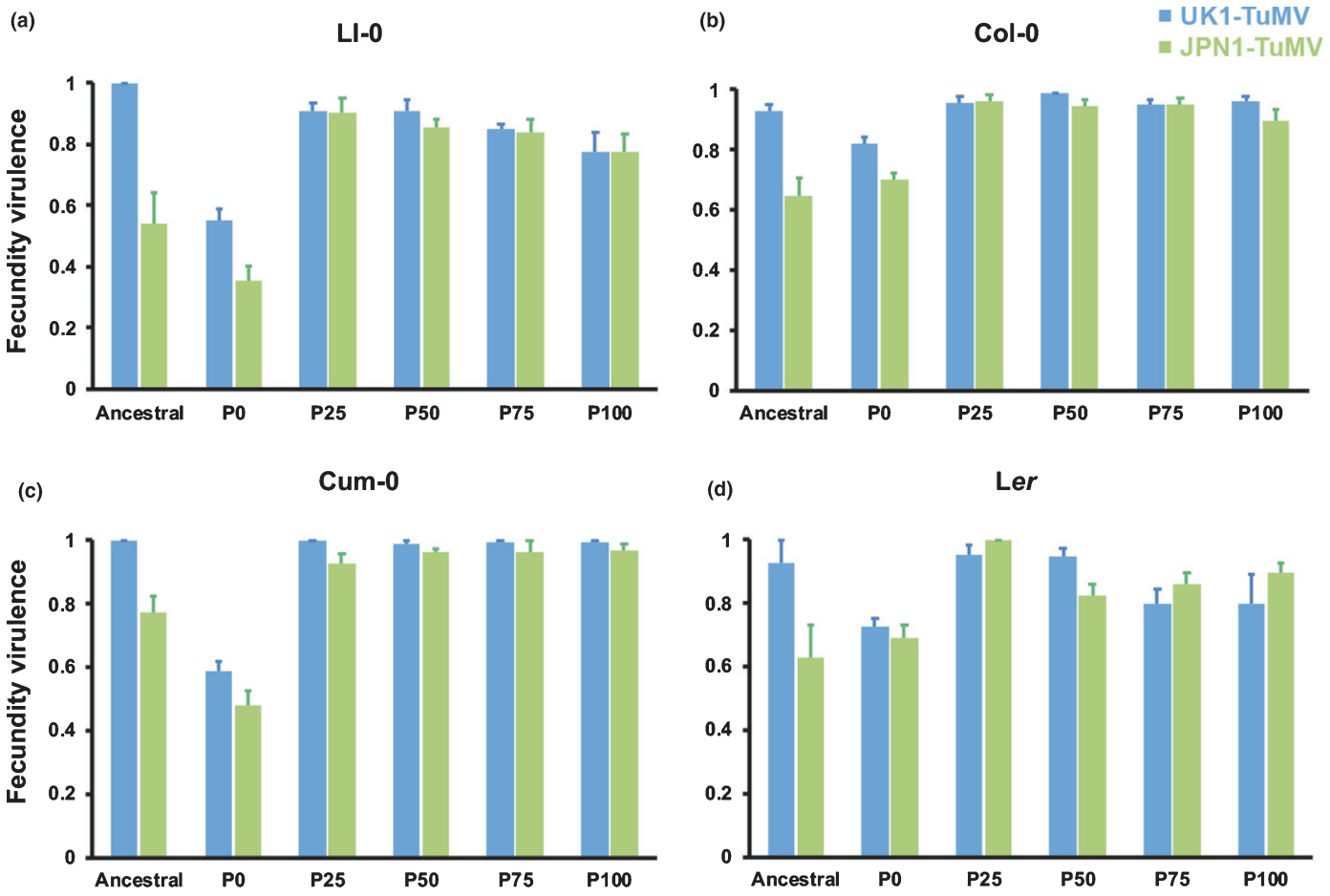

Fig. 4 TuMV fecundity virulence in *Arabidopsis thaliana* genotypes. Effect of virus infection on plant seed weight ($1 - (SW_i/SW_m)$) in Ll-0 (a), Col-0 (b), Cum-0 (c) and L*er* (d). Data are given as mean ± SE of seven replicates per three lineages. TuMV isolates: UK1-TuMV (blue), JPN1-TuMV (green). Ancestral, P0, P25, P50, P75 and P100 refers to the host population from which the virus was obtained.

JPN1-TuMV fecundity virulence also differed between the ancestral and the passaged viruses in all genotypes (Wald's test $\chi^2 \geq 61.04$, $P < 1 \times 10^{-4}$) (Fig. 4): Passaged viruses had higher virulence than the ancestral ($P \leq 0.041$), with the exception of P0 viruses that showed the opposite trend in Ll-0 and Cum-0 ($P \leq 0.052$) and no differences with the ancestral in Col-0 and L*er* ($P \geq 0.299$). Fecundity virulence of P25 to P100 viruses was always similar ($P \geq 0.080$), except for L*er* in which P25 showed higher virulence than the other passaged viruses ($P \leq 0.031$). Host genotype and population structure explained a small fraction of the variance in fecundity virulence (2% and 7%, respectively). Also, JPN1-TuMV fecundity virulence was similar in plant genotypes that did or did not conform to host populations in which the viruses were passaged (Wald's test $\chi^2_{1,410} = 2.01$, $P = 0.157$).

In summary, the evolution of TuMV fecundity virulence depended on the virus isolate. For the highly virulent UK1-TuMV, serial passaging generally had little effect on virulence. Conversely, serial passaging of the less virulent JPN1-TuMV generally increased fecundity virulence. Exceptions were viruses serially passaged in P0 populations, which mostly showed reduced virulence.

Effect of TuMV evolution on Arabidopsis mortality tolerance

The results above indicated that host population structure influenced the evolution of virus multiplication and virulence. Because tolerance is quantified by the relationship between these two traits, we analysed if virus evolution resulted in changes in plant mortality tolerance.

In all four genotypes, Arabidopsis mortality tolerance (the slope of the *LP* to virus multiplication relationship) to UK1-TuMV differed between viruses (Wald's test $\chi^2 \geq 6.66$, $P \leq 7.3 \times 10^{-3}$) (Fig. 5). In Cum-0 and Col-0, mortality tolerance to P0, P25 and P50 viruses was generally similar to tolerance to the ancestral virus ($P \geq 0.170$); whereas tolerance to P75 and P100 viruses was higher (shallower slope) ($P \leq 0.032$). Exception were Col-0 plants, which showed tolerance to P0 in the same range as to P75 and P100 viruses ($P \geq 0.218$), and higher than tolerance to the ancestral virus ($P = 0.002$). Ll-0 and L*er* plants showed higher tolerance to all passaged viruses than to the ancestral ($P \leq 1 \times 10^{-4}$) and, as for the other two genotypes, tolerance to P0, P25 and P50 was lower (steeper slope) than to the P75 and P100 viruses ($P \leq 0.050$) (Fig. 5). Host population structure

explained a larger proportion of the variance in mortality tolerance than did the plant genotype (46% and 17%, respectively). The presence/absence of a genotype in the plant populations in which viruses were passaged did not affect mortality tolerance (Wald's test $\chi^2_{1,46} = 0.63$, $P = 0.681$).

In JPN1-TuMV-infected plants, there was again a general trend towards higher mortality tolerance to viruses that evolved in populations with a larger proportion of MT plants (Wald's test $\chi^2 = 5.59$, $P \leq 0.013$) (Fig. 5). Ll-0, Col-0 and L*er* showed lower tolerance to the ancestral than to the passaged viruses ($P \leq 0.038$), except for P0 viruses in L*er*, and P25 viruses in Col-0 and L*er*, for which tolerance was similar to that found for the ancestral virus ($P \geq 0.307$). In these three plant genotypes, mortality tolerance was higher to P75 and P100 viruses than to the other viruses (Fig. 5). Although Cum-0 plants also tended to display higher tolerance to P75 and P100 viruses, differences were not significant ($P \geq 0.283$), except for P25 viruses to whom plants were much less tolerant than to any other virus ($P = 3 \times 10^{-4}$). Host genotype and population structure explained little of the variance in mortality virulence (12% each). However, these results were just due to the great variability in Cum-0. When this genotype was removed, host population structure explained a much larger proportion of the variance in mortality tolerance than plant genotype (62% and 2%, respectively).

Therefore, Arabidopsis plants had generally higher mortality tolerance to viruses evolved in populations with a larger percentage of more tolerant plants.

Effect of TuMV evolution on Arabidopsis fecundity tolerance

We also analysed fecundity tolerance as the slope of *SW* versus virus accumulation relationship. For UK1-TuMV, fecundity tolerance to each virus differed in Ll-0, Col-0 and L*er* (Wald's test $\chi^2 \geq 5.60$, $P \leq 0.010$) (Fig. 6). These three genotypes had generally higher fecundity tolerance to P50, P75 and P100 viruses than to the ancestral virus and to P0 and P25 viruses ($P \leq 0.029$), with tolerance to the later three viruses being similar ($P \geq 0.302$). Exceptions were Col-0 plants, which showed a significantly lower fecundity tolerance to P0 than to any other virus ($P = 1 \times 10^{-4}$). Again, although Cum-0 exhibited the same trend as the other genotypes, observed variation in fecundity tolerance to the different viruses was not significant (Wald's test $\chi^2_{5,8} = 2.48$, $P = 0.122$) (Fig. 6). Host population structure explained a larger proportion of the variance in fecundity tolerance than the

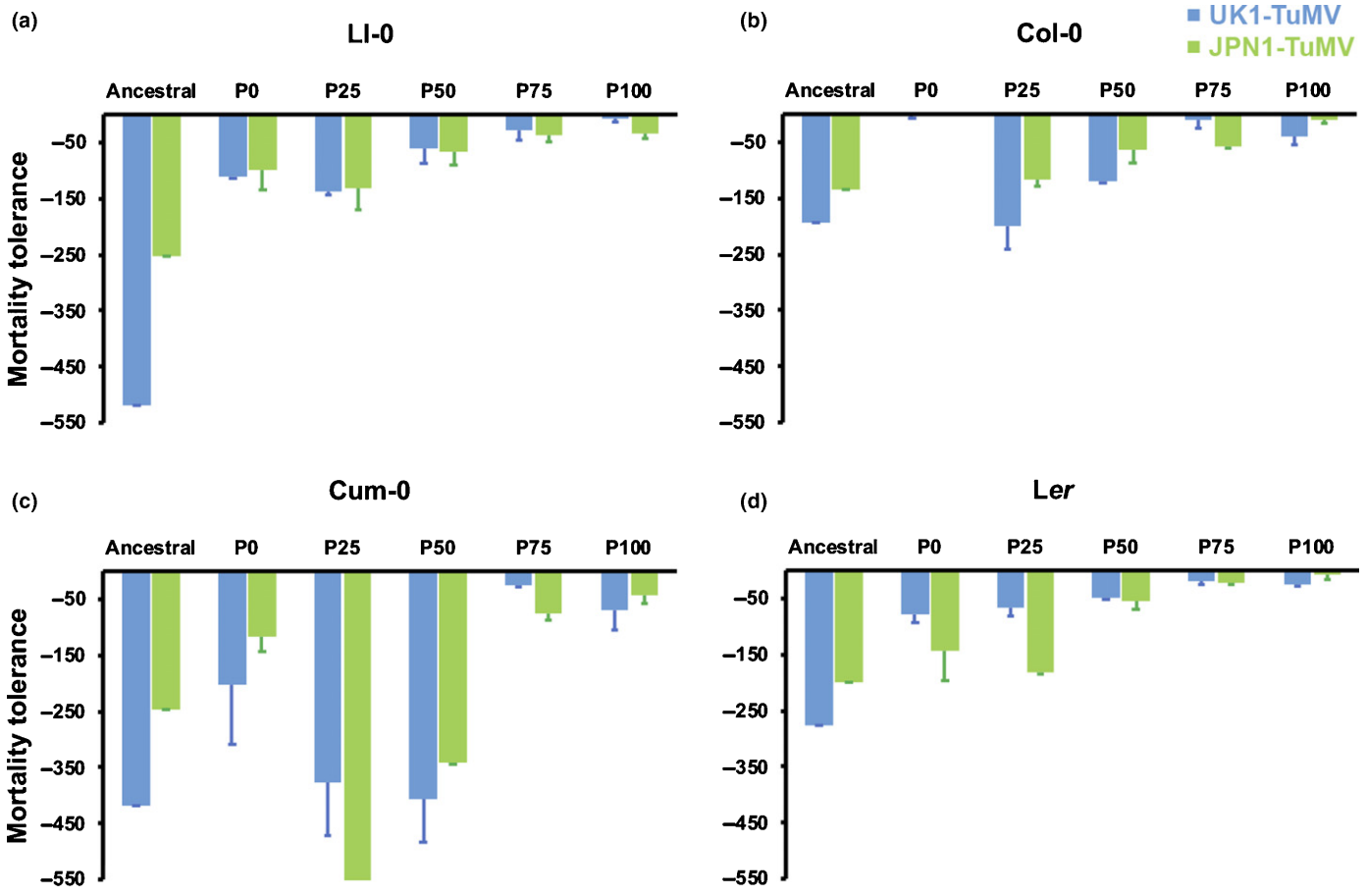

Fig. 5 TuMV mortality tolerance in *Arabidopsis thaliana* genotypes. Slope of the *LP* to virus accumulation relationship in Ll-0 (a), Col-0 (b), Cum-0 (c) and L*er* (d). Data are given as mean ± SE of seven replicates of three lineages. TuMV isolates: UK1-TuMV (blue), JPN1-TuMV (green). Ancestral, P0, P25, P50, P75 and P100 refers to the host population from which the virus was obtained.

genotype (27% vs 17%, respectively), and host genotype-specific adaptation had no effect (1%).

Similarly, trends were observed when plants were infected with JPN1-TuMV (Fig. 6), with Ll-0, Col-0 and L*er* plants showing a significantly higher fecundity tolerance to P50, P75 and P100 viruses than to the other viruses ($P \leq 0.045$). All three genotypes were equally tolerant to P25 viruses ($P \geq 0.123$), and Col-0 and L*er* were significantly less tolerant to P0 viruses ($P \leq 0.040$), than to the ancestral virus (Fig. 6). Host genotype explained a smaller proportion of the variance than the population structure (15% vs 33%).

These results indicated that virus evolution resulted in changes in plant fecundity tolerance, with plants being generally more tolerant to viruses that had evolved in host populations with larger proportions of tolerant genotypes.

Relationship between virus multiplication, virulence and plant tolerance

As we observed changes in all traits in response to plant population structure for tolerance, which depended on the virus isolate × plant–virus interaction; and theoretical models predicted that these changes were associated with them (see Introduction), we analysed these relationships for each virus and plant genotype separately (Fig. 7). We found a positive nonlinear association between virus multiplication and both fecundity ($R^2 \geq 0.39$; $P \leq 0.009$) and mortality ($R^2 \geq 0.25$; $P \leq 0.056$) tolerances (Fig. 7a–h). In addition, mortality virulence was negatively associated with mortality tolerance ($R^2 \geq 0.27$; $P \leq 0.055$) (Fig. 7i–l). Finally, both fecundity and mortality virulence ($R^2 \geq 0.24$; $P \leq 0.050$) (Fig. 7m–p), and fecundity and mortality tolerance ($R^2 \geq 0.41$; $P \leq 0.010$) (Fig. 7q–t), were also positively associated. No significant association was found for any other pairwise combination of the five traits ($R^2 \leq 0.37$; $P \geq 0.074$).

Comparison of JPN1-TuMV and UK1-TuMV evolution

Finally, we explored whether both viruses, which initially differed in multiplication and virulence in Arabidopsis, evolved towards a common optimal solution or maintained original differences. To do so, we compared the evolution of accumulation, virulence and host tolerance between UK1-TuMV and JPN1-TuMV. In the four Arabidopsis genotypes, virus accumulation and virulence, and host tolerance, significantly differed between UK1-TuMV

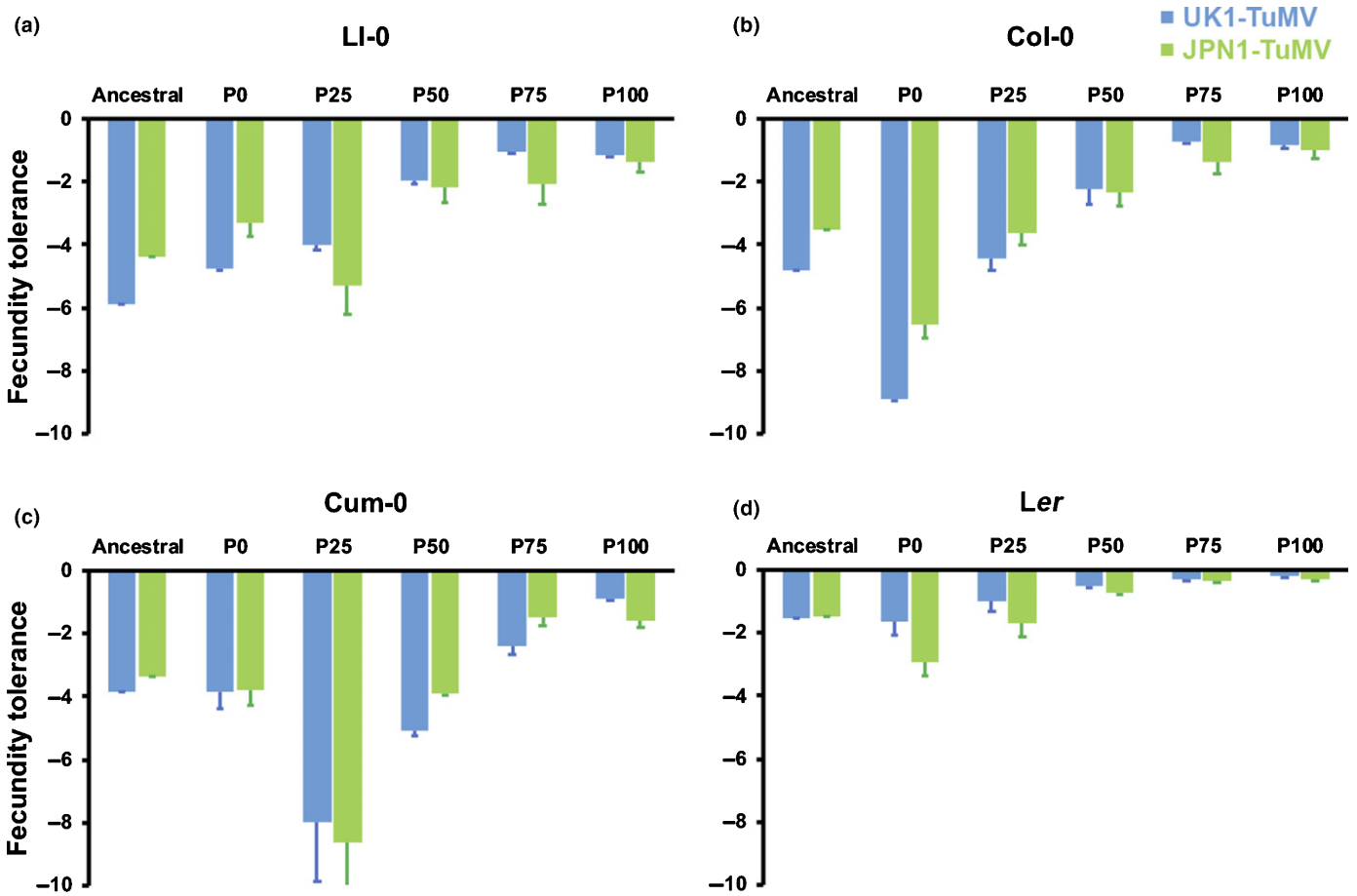

Fig. 6 TuMV fecundity tolerance in *Arabidopsis thaliana* genotypes. Slope of the *SW* to virus accumulation relationship in Ll-0 (a), Col-0 (b), Cum-0 (c) and Ler (d). Data are given as mean ± SE of seven replicates per three lineages. TuMV isolates: UK1-TuMV (blue), JPN1-TuMV (green). Ancestral, P0, P25, P50, P75 and P100 refers to the host population from which the virus was obtained.

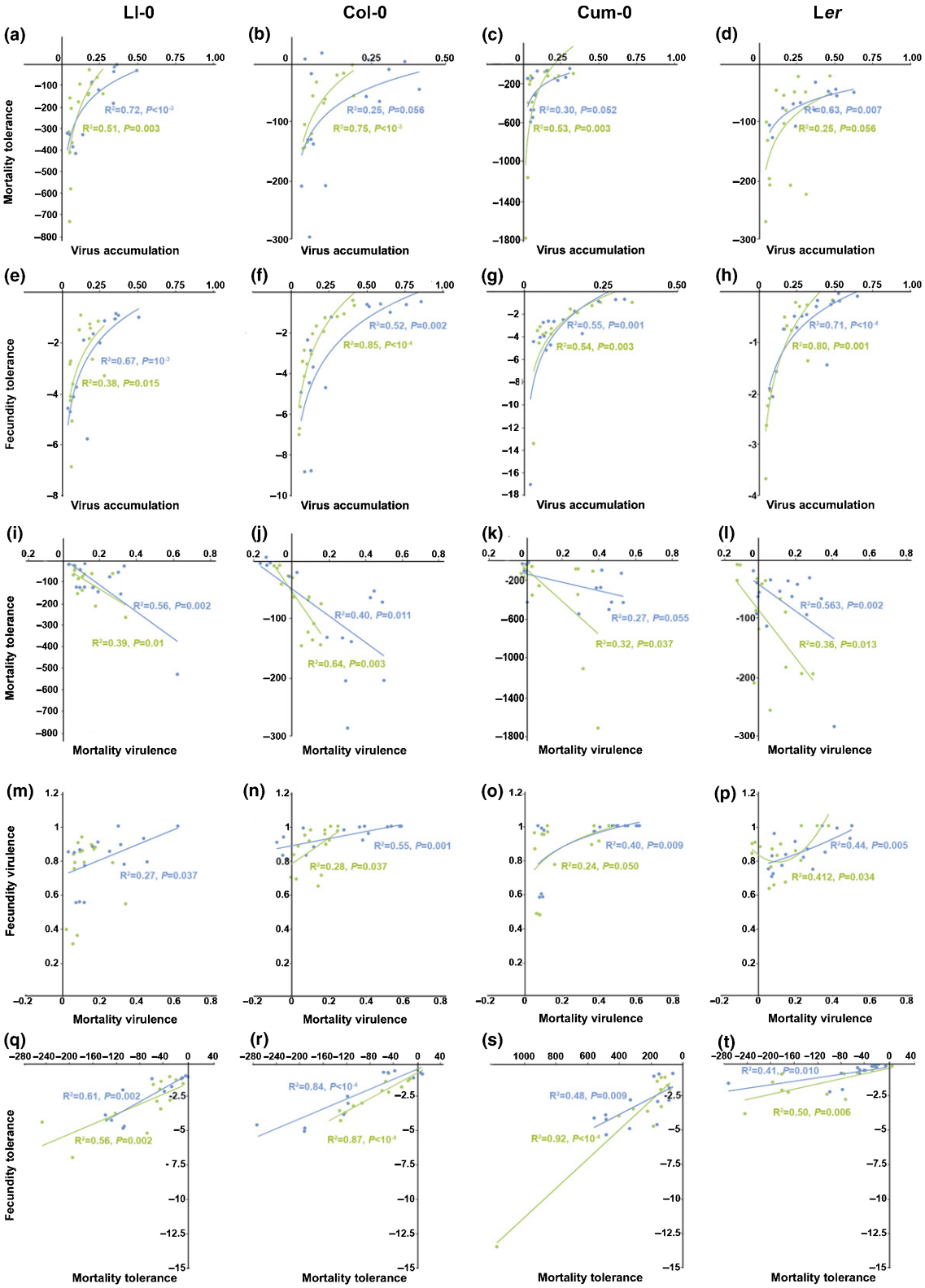

Fig. 7 Relationships between TuMV infection traits in *Arabidopsis thaliana* genotypes. (a–d) Bivariate relationship between virus multiplication and mortality tolerance. (e–h) Bivariate relationship between virus multiplication and fecundity tolerance. (i–l) Bivariate relationship between mortality tolerance and mortality virulence. (m–p) Bivariate relationship between fecundity virulence and mortality virulence. (q–t) Bivariate relationship between fecundity tolerance and mortality tolerance. TuMV isolates: UK1-TuMV (blue), JPN1-TuMV (green). Note the different scales in the *x*-axis and *y*-axis depending on the combination of variables. Units are displayed as shown in Figs 2–6. Data correspond to average values of the three lineages evolved in each host population plus the ancestral.

and JPN1-TuMV ancestors (Wald's test $\chi^2 \geq 5.40$, $P \leq 0.020$). In addition, accumulation of P0, P25 and P50 viruses derived from both isolates was generally similar in all plant genotypes (Wald's test $\chi^2 \leq 2.88$, $P \geq 0.090$), and generally differed for P75 and P100 viruses (Wald's test $\chi^2 \geq 3.45$, $P \leq 0.063$). Similarly, mortality virulence did not generally differ between UK1-TuMV and JPN1-TuMV for all (Wald's test $\chi^2 \geq 2.90$, $P \leq 0.089$), except for P100 (Wald's test $\chi^2 \geq 10.35$, $P \leq 0.001$) viruses. Also, viruses that had evolved from both ancestors in a given host population structure generally showed similar values of fecundity virulence (Wald's test $\chi^2 \leq 2.81$, $P \geq 0.094$). By contrast, fecundity and mortality tolerances to passaged UK1-TuMV and JPN1-TuMV were not similar in any case (Wald's test $\chi^2 \geq 3.27$, $P \leq 0.044$).

Therefore, UK1-TuMV and JPN1-TuMV passaged viruses generally converged towards similar values of accumulation, and of mortality and fecundity virulence, with the exception of viruses passaged in P100 populations.

Discussion

As tolerance was conceptually defined more than a century ago (Cobbs, 1894), evidence on the importance of this mechanisms as a plant defence against pathogens has steadily increased (Pagán & García-Arenal, 2020). However, little information is known of the effect of population structure for tolerance on pathogen evolution and how this affects the maintenance of host tolerance, and to date most of the work on this subject is restricted to mathematical modelling. We experimentally tested the most relevant predictions of these theoretical works for a plant–virus interaction: (1) pathogen multiplication increases, and virulence decreases, at higher host mortality tolerance; and (2) pathogen multiplication also increases with fecundity tolerance, whereas this defence is maximised at intermediate levels of mortality virulence.

TuMV evolution in Arabidopsis populations with a higher proportion of plants that were tolerant to virus-induced mortality resulted in increased virus multiplication. According to theory, in host populations with higher proportions of tolerant individuals more exploitative pathogens would be favoured (Miller *et al.*, 2006; van den Bosch *et al.*, 2006, 2007). In our experiments, we pooled equal amounts of tissue from each plant of a given host population as the inoculum for the next serial passage, and we minimised the effects of transmission bottlenecks by inoculating at saturation. Under these conditions, transmission rate positively correlated with virus within-host multiplication, simulating an association that has been repeatedly reported for potyviruses regardless of whether transmission was through mechanical inoculation or by aphids (e.g. Froissart *et al.*, 2010; Hajimorad *et al.*, 2011). Therefore, in tolerant plants, viruses that achieve higher multiplication levels, and that do not have the limitation of paying the cost of killing the plant before sampling time, increase their chances to be transmitted. This selective advantage is compatible with our results and agrees with the few previous experimental works showing a trend towards higher virus multiplication in tolerant host populations (Cronin *et al.*, 2014; Vijayan *et al.*, 2017). Moreover, such evolution towards higher multiplication is generally predicted under a negative linear correlation between mortality virulence and mortality tolerance, a condition met by our experimental system. It should be noted that some of the models covering general host–pathogen theory are constructed assuming direct pathogen transmission. This is not the case of most plant viruses, which are dispersed by vectors. Zeilinger & Dougherty (2014) included vector preference to model the consequences of plant tolerance for the host and the virus population, finding that tolerance increases virus transmission (and therefore promotes higher virus multiplication) only if it does not result in less attractive plants for vectors. Therefore, models developed for hosts and parasites in general and for plants and viruses in particular reached the same conclusions only if more tolerant hosts had the same (or more) attractiveness for the vector. Notably, TuMV evolution in tolerant Arabidopsis results in more intense yellow mosaic patterns (Vijayan *et al.*, 2017), a trait known to increase aphid attraction (Salvaudon *et al.*, 2013). This would explain why our results supported predictions of both types of models.

By contrast, viruses passaged in less tolerant plant populations tended to reduce their multiplication. In the absence of tolerance, pathogens (including viruses) play with different rules. The most accepted view is that higher pathogen multiplication increases between-host transmission but also virulence, which reduces the infectious period and therefore transmission. Therefore, pathogens would evolve towards intermediate levels of within-host multiplication and mortality virulence to optimise transmission, in what is called the trade-off hypothesis (Anderson & May, 1982; Alizon *et al.*, 2009). In less tolerant Arabidopsis populations, the only way for a highly virulent virus, such as TuMV, to be transmitted in the conditions of our experiment was reducing host mortality to the point of allowing plant survival until sample collection, which according to the trade-off hypothesis would explain the observed reduction in virus multiplication. This would be particularly so for lineages derived from UK1-TuMV, a highly virulent isolate whose infection induces early plant senescence (Montes *et al.*, 2020). In agreement, the reduction of JPN1-TuMV (a less virulent isolate, Vijayan *et al.*, 2017) multiplication after passaging in less tolerant plants was milder than for UK1-TuMV.

All the rationale above is based on the existence of a positive relationship between virus multiplication and mortality virulence. Although this is a general assumption of the trade-off hypothesis (Anderson & May, 1982; Alizon *et al.*, 2009) and of mathematical models predicting the evolution of pathogens in response to tolerance (Miller *et al.*, 2005, 2006; van den Bosch *et al.*, 2006; Cronin *et al.*, 2014), experimentation with plant viruses often did not find such a link (Escriu *et al.*, 2003; Stewart *et al.*, 2005; Pagán *et al.*, 2007, 2008). Our results, however, provide two lines of evidence supporting an association between TuMV multiplication and Arabidopsis mortality. First, bivariate analyses indicated a general negative correlation between Arabidopsis lifespan and TuMV load (if not, tolerance could not be quantified as the slope of this relationship). This correlation was observed for every host-virus genotype per genotype interaction,

indicating that it is a general property of the pathosystem in agreement with our previous work (Montes & Pagán, 2019; Montes *et al.*, 2020). Second, changes in TuMV mortality virulence after serial passages fitted with mathematical models predicting evolution towards lower levels, but the degree of change depended on the plant population structure for tolerance. On the one hand, despite the increased multiplication of viruses that evolved in more tolerant plants, their mortality virulence was lower than that of the ancestral virus, which is compatible with no cost in transmission for more exploitative viruses. On the other hand, the drastic reduction in multiplication of viruses that evolved in less tolerant plants was accompanied by a similar decrease in mortality virulence, which suggests that in the absence of tolerance these two traits remained coupled and needed to be simultaneously reduced to optimise transmission. In agreement, changes in JPN1-TuMV mortality virulence were less pronounced than for UK1-TuMV, mimicking the evolution of virus multiplication. The strong selection towards lower virus multiplication and virulence exerted by less tolerant hosts is also reflected by the convergent evolution of the two virus isolates in both traits when passaged in less tolerant, but not in more tolerant, populations.

The evolution of TuMV mortality virulence contrasted with changes in virus-induced reduction of host fecundity. Serial passages of JPN1-TuMV in Arabidopsis generally increased fecundity virulence to almost prevent seed production, and resembling UK1-TuMV infection. Two hypotheses may explain these results:

(**1**) Because we maintained a constant population size and tolerance structure across serial passages, plant progeny production had no role on plant and virus fitness (i.e. lower seed production did not cause host depletion). Therefore, the observed patterns are just due to neutral evolution (Best *et al.*, 2008).

(**2**) Changes in fecundity virulence promote transmission and are selectively advantageous for the virus. TuMV generally prevents Arabidopsis reproduction (Sánchez *et al.*, 2015; Vijayan *et al.*, 2017).

In this context, hosts may redistribute resources towards plant survival by enlarging the infectious period (Obrebski, 1975; Lafferty & Kuris, 2009), and to host growth when resources become available for pathogen multiplication (Ebert *et al.*, 2004). Modifications of the trade-off hypothesis to accommodate pathogens that block host reproduction predict that the lower the host progeny production and mortality, the higher the transmission rate (O'Keefe & Antonovics, 2002; Ebert *et al.*, 2004; Hall *et al.*, 2007). Notably, these predictions fit with the observed patterns of TuMV evolution: host fecundity near zero was linked to reduced mortality virulence and, in many cases, to higher virus multiplication. Moreover, convergent evolution of both isolates towards similar levels of fecundity virulence suggested that selection rather than neutral evolution played a role in the observed patterns. Therefore, our data support the idea that TuMV evolved fecundity virulence to optimise transmission. In addition, dropping of virus multiplication after passages in less fecundity tolerant populations also supported theoretical predictions on the evolution of castrating pathogens (Best *et al.*, 2010). Indeed, these results agreed with the behaviour of other plant castrators (Clay, 1991; Kover, 2000), and with JPN1-TuMV evolution in genetically homogeneous Arabidopsis populations (Vijayan *et al.*, 2017). Interestingly, these authors sequenced the TuMV genome after serial passaging, mapping clusters of mutations in proteins controlling virus multiplication, symptom development and within-host movement. Although here we did not sequence viral progenies, it is likely that mutations in the same genes could be mapped at least for P100 viruses.

The plant population structure-driven evolution of TuMV multiplication and virulence also resulted in modifications of Arabidopsis mortality and fecundity tolerance, suggesting feedback loops between plant tolerance and virus evolution. Plants showed higher mortality and fecundity tolerance to viruses passaged in more tolerant populations than to the ancestral or to those passaged in less tolerant hosts. These results fit with the observed changes in other infection traits: as tolerance was measured as plant fitness reduction per unit of virus load (Little *et al.*, 2010; Zeilinger & Daugherty, 2014), the increase of multiplication and the reduction of virulence displayed by P100 viruses compared with the ancestral virus will result in a shallower slope of the relationship between these two traits. Conversely, large reductions of both multiplication and virulence in P0 viruses is likely to result in lesser modifications of tolerance. Despite higher mortality and fecundity tolerance, fitness of plants infected by P100 viruses was much lower than for uninfected controls, indicating that no absolute tolerance was achieved (Cooper & Jones, 1983). Then, it could be argued that this level of fecundity tolerance is not effective (Shuckla *et al.*, 2018). Certainly, it will be of little use from an agronomic perspective, but it may be selectively advantageous in wild ecosystems: as this level of fecundity tolerance allows plants to produce progeny at a level of virus multiplication that in less tolerant genotypes prevents seed production (Montes *et al.*, 2020), it makes the difference between leaving progeny or not. Indeed, various models have shown that this level of fecundity tolerance drives the host population out of the pathogen-driven extinction margins, especially at high levels of pathogen prevalence (Boots & Sasaki, 2002; Antonovics, 2009).

Interestingly, maximal tolerance was observed at intermediate levels of mortality virulence, particularly for UK1-TuMV viruses, as predicted by mathematical models (Fornoni *et al.*, 2004; Best *et al.*, 2010). These models were constructed under the assumption that tolerance is a host-controlled trait, which in Arabidopsis–virus interactions is supported by experimental evidence showing that this trait has medium–high plant heritability (Pagán *et al.*, 2007, 2008; Montes *et al.*, 2019, 2020). Still, in these experimental works part of the variance in tolerance remained unexplained. Our results indicated that at least part of this unassigned variance is accounted for by the virus. Moreover, our work showed that tolerance is controlled by the plant per virus genotype × genotype interaction, indicating that it is genetically controlled by both partners. Therefore, we propose that tolerance can be viewed as a property of the plant–virus interaction (and therefore as much a consequence of plant as of virus traits). This expanded view of tolerance has been theoretically considered (Boots & Bowers, 1999; Vitale & Best, 2019), but remains

experimentally unexplored. Such analyses can be only addressed through the joint effort of plant biologist and virologists and is an interesting venue for future research.

Our work also provides insights into two additional aspects on the evolutionary dynamics of hosts and pathogens in the context of tolerance. The first relates to the relationship between tolerance and other defence mechanisms such as resistance. Mathematical models on the evolution of defence strategies against pathogens generally accommodate a trade-off between resistance and tolerance such that host fitness is maximised at maximum tolerance or maximum resistance (Mauricio & Rausher, 1997; Boots & Bowers, 1999) or at intermediate levels of both (Restif & Koella, 2003, 2004; Fornoni *et al.*, 2004) depending on the costs of each defence strategy for the host. Our results provide support for these predictions. First, viruses passaged in less tolerant hosts evolved minimal multiplication (maximal resistance) and those adapted to tolerant hosts evolved maximal tolerance, with polymorphisms for tolerance leading to intermediate levels of both defence traits. Second, our bivariate analyses detected a positive association between virus multiplication and fecundity and mortality tolerance, and therefore a negative association between resistance and tolerance. Therefore, our results showed that virus (and not only host) evolution may also favour one or the other plant defence to avoid extinction and/or maximise transmission, again pointing to a shared control of these traits. The second aspect relates to other general prediction of theoretical models: our analysis in less tolerant hosts infected by viruses evolved in more tolerant populations that allowed testing the prediction that pathogen evolution in a tolerant host results in higher virulence than in more susceptible ones. In general, virulence of viruses passaged in fully tolerant host populations was similar in both more and less tolerant genotypes. Indeed, comparisons of P100 mortality and fecundity virulence between Col-0 and L*er* (MT) vs Cum-0 and Ll-0 (LT) showed no significant differences in these traits (Wald's test $\chi^2 \leq 0.42$, $P \geq 0.518$), indicating that the prediction of theoretical models does not hold in our experimental system. Similarly, in a previous study on virus evolution in response to plant absolute tolerance, deployment of zucchini squash varieties tolerant to ZYMV resulted in virus evolution towards higher aggressiveness in the initially tolerant varieties, such that tolerant and nontolerant varieties could not be differentiated according to the symptoms induced by the evolved ZYMV strain. These aggressive virus strains also induced severe fruit malformations, indicative of higher virulence (Desbiez *et al.*, 2003). In our experimental design, which only allowed virus evolution as in Desbiez *et al.* (2003) and as in most agricultural settings, we also observed increased fecundity virulence in tolerant plants infected by viruses passaged in this type of hosts, particularly those derived from JPN1-TuMV. Therefore, ours and previous results provide evidence of the potential risks of deploying tolerance (even if it not absolute) as a strategy to control plant diseases.

In summary, our analyses support most, but not all, theoretical predictions on pathogen evolution in response to host tolerance. We also showed that the host population structure for tolerance determines how viruses tune the relationship between multiplication and virulence to optimise transmission, which in turn feeds back on plant tolerance. Altogether, this work provides compelling evidence that tolerance is modulated by the plant per virus interaction.

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Author contributions

IP designed the research, NM analysed the data, VV performed the experiments, IP and NM wrote the manuscript.

ORCID

Nuria Montes https://orcid.org/0000-0001-9526-2772
Israel Pagán https://orcid.org/0000-0001-8876-1194

Data availability

Data available in supplementary material.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Dataset S1 Raw data generated in this work.

Notes S1 Examples of R code used for statistical analyses.

Table S1 Mean, minimum and maximum values of virus accumulation, effect of virus infection on plant lifespan and progeny production and plant fecundity and mortality tolerance of each Arabidopsis genotype and TuMV lineage.

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