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Neopolyploidy and pathogen resistance

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Despite the well-documented historical importance of polyploidy, the mechanisms responsible for the establishment and evolutionary success of novel polyploid lineages remain unresolved. One possibility, which has not been previously evaluated theoretically, is that novel polyploid lineages are initially more resistant to pathogens than the diploid progenitor species. Here, we explore this possibility by developing and analysing mathematical models of interactions between newly formed polyploid lineages and their pathogens. We find that for the genetic mechanisms of pathogen resistance with the best empirical support, newly formed polyploid populations of hosts are expected to be more resistant than their diploid progenitors. This effect can be quite strong and, in the case of perennial species with recurrent polyploid formation, may last indefinitely, potentially providing a general explanation for the successful establishment of novel polyploid lineages.

Keywords: polyploidy; evolution; parasite; coevolution; establishment; minority cytotype exclusion

1. INTRODUCTION

The evolutionary success of polyploid plants (Masterson 1994; Otto & Whitton 2000) is striking, given the hurdles posed to their establishment by minority cytotype exclusion (Levin 1975; Husband 2000) and inevitable competition with more abundant diploid ancestors (Baack 2005; Rausch *et al.* 2005). Existing theory has identified several possible explanations for the remarkable success of polyploids, including temporary masking of deleterious alleles, and an increased rate of adaptation when new beneficial mutations are dominant (Otto & Whitton 2000). However, the generality of these advantages is questionable because increased masking of deleterious mutations also leads to an increase in their frequency (Otto & Whitton 2000), and little data are available on the dominance of new beneficial mutations (Bagheri & Wagner 2004).

Another possibility, originally suggested by Levin (1983), is that newly formed polyploid lineages are more resistant to pathogens than are their diploid progenitors. Empirical studies have evaluated this possibility with mixed results. Specifically, these studies have shown tetraploids to be more resistant in some cases, but less resistant in others (Burdon & Marshall 1981; Schoen *et al.* 1992; Busey *et al.* 1993; Guegan & Morand 1996; Thompson *et al.* 1997; Nuismer & Thompson 2001). However, these results are based on only one or a few independent polyploid lineages and thus do not provide a general picture of the level of pathogen resistance expected in newly formed polyploid populations. Consequently, it is currently not possible to evaluate the role interactions with pathogens play in promoting the establishment of polyploidy lineages.

In order to rigorously evaluate whether neopolyploids should, in general, be more resistant than their diploid progenitors, we used three genetic models of pathogen resistance. These models considered a diverse range of empirically supported genetic mechanisms of pathogen resistance including the gene-for-gene (GFG), inverse matching alleles (IMA) and matching alleles (MA) models. The GFG interaction is thought to underlie many host–pathogen interactions (Burdon 1987; Thompson & Burdon 1992; Hammond-Kosack & Jones 1997). In this model, host resistance alleles are generally dominant and pathogen virulence alleles are generally recessive (Agrawal & Lively 2002). GFG interactions are particularly well documented in interactions between plants and pathogens (Burdon 1987), making this an exceptionally important model for understanding the evolutionary consequences of parasitism for novel polyploid lineages. The IMA model is based upon the idea that hosts can mount an immune response against only those pathogens carrying alleles recognized by specific host alleles, as is the case for the vertebrate major histocompatability complex (MHC) system (Singh & Krimbas 2000). Finally, the MA model is based upon a system of self/nonself recognition (Grosberg & Hart 2000) where hosts can recognize and mount an immune response against only those pathogens carrying alleles different from self-alleles (Agrawal & Lively 2002). By extending these three genetic models, we were able to examine in detail the immediate effects of polyploidy on pathogen resistance using analytic methods and computer simulations.

2. MATERIAL AND METHODS

(a) *Analytic*

Our general approach can be illustrated using the GFG model and a single diallelic locus as an example. By assuming hosts encounter parasites at random, the expected proportion of resistant hosts can be calculated by summing the probability of host resistance shown in table 1 across all possible combinations of host and pathogen genotypes. However, summing the infected terms and subtracting from unity is more tractable when dealing with multiple alleles, so we take this approach in our example. Using this method, the expected

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Table 1. Infection matrix for the case of a single diallelic locus. ('I' represents infection of the host by the pathogen and 'R' resistance to the pathogen for the GFG (red), MA (green) and IMA (blue) models, respectively. Genotype frequencies in the diploid host and pathogen are as expected under Hardy–Weinburg equilibrium. Tetraploid host genotype frequencies are derived assuming random combination of unreduced gametes from the diploid population.)

		diploid host			tetraploid host				
	genotype frequency	A_1A_1 p_{h1}^2	A_1A_2 $2p_{h1}p_{h2}$	A_2A_2 p_{h2}^2	$A_1A_1A_1A_1$ p_{h1}^4	$A_1A_1A_1A_2$ $4p_{h1}^3p_{h2}$	$A_1A_1A_2A_2$ $6p_{h1}^2p_{h2}^2$	$A_1A_2A_2A_2$ $4p_{h1}p_{h2}^3$	$A_2A_2A_2A_2$ p_{h2}^4
pathogen	A_1A_1 p_{p1}^2	I,I,R	R,I,R	R,R,I	I,I,R	R,I,R	R,I,R	R,I,R	R,R,I
	A_1A_2 $2p_{p1}p_{p2}$	I,R,R	R,I,R	R,R,R	I,R,R	R,I,R	R,I,R	R,I,R	R,R,R
	A_2A_2 p_{p2}^2	I,R,I	I,I,R	I,I,R	I,R,I	I,I,R	I,I,R	I,I,R	I,I,R

proportion of resistant diploids and autotetraploids is

$$\overset{\text{GFG}}{R}_2 = 1-\left(p_{p1}^2p_{h1}^2-2p_{p1}p_{p2}p_{h1}^2-p_{p2}^2\right) \quad \text{and,} \tag{2.1a}$$

$$\overset{\text{GFG}}{R}_4 = 1-\left(p_{p1}^2p_{h1}^4-2p_{p1}p_{p2}p_{h1}^4-p_{p2}^2\right), \tag{2.1b}$$

respectively, where p_{p1} and p_{p2} are the frequencies of the A_1 and A_2 alleles in the pathogen, and p_{h1} and p_{h2} are the frequencies of the A_1 and A_2 alleles in the host. This approach can be readily extended to any number of alleles and to multiple diallelic loci (electronic supplementary material, methods).

(b) *Simulations*

Individual-based simulations written in Java were initiated with 1, 5 or 10 tetraploid individuals with lifespans of 1, 5 or 10 years created by the union of unreduced gametes drawn from a simulated diploid population of 2000 individuals. These tetraploid individuals mated at random and produced a single offspring in the last year of their lives. Consequently, the population size was assumed to be fixed with the exception of new tetraploid individuals formed by the union of unreduced gametes, which was assumed to occur at a rate of μ per year. Simulations assumed that one or two loci, and two or four alleles moderated the host–pathogen interaction. When multiple loci were incorporated, we assumed free recombination. Random mating was simulated by forming a large gamete pool and forming new individuals by random combination of gametes (without replacement). These simulations were replicated 500 times and the mean difference in resistance across ploidies was calculated for each of 50 generations.

3. RESULTS AND DISCUSSION

For each genetic mechanism of resistance, we compared the expected level of pathogen resistance in the diploid population to that in the newly formed autotetraploid population. We initially assumed that resistance was determined by a single locus with an arbitrary number of alleles and that both the diploid host population and the parasite population were at Hardy–Weinberg equilibrium. In all cases, the novel autotetraploid population was assumed to arise from the random union of unreduced diploid gametes, a common mode of polyploid formation in natural populations (Bretagnolle & Thompson 1995; Otto & Whitton 2000). These assumptions allowed us to derive simple expressions for the expected level of pathogen resistance in the diploid and tetraploid populations (see §2 and electronic supplementary material). We found that in the case of the GFG and IMA models, the newly formed autotetraploid population will always be more resistant than the diploid population from which it arose. In contrast to the GFG and IMA models, a similar derivation for the MA model shows that the diploid population is always more resistant. The MA model is, however, the genetic model of resistance with the least empirical support, particularly for the case of plant–pathogen interactions considered here (Agrawal & Lively 2002).

To this point, our results demonstrate that differences in resistance always exist between the progenitor diploid and novel autotetraploid population, but that the magnitude of these differences depends on initial allele frequencies within the diploid population. In order to visually represent the expected magnitude of the difference in resistance, we assumed that the host and pathogen allele frequencies were normally distributed which reduced dimensionality sufficiently to plot differences in resistance for various allele frequency distributions (figure 1). We found that the difference in resistance between ploidies is quite large over much of the parameter space, particularly when the variance of the distributions is large. A large variance corresponds to the case where resistance/infection alleles occur with similar frequencies, as is the case in some well-studied systems such as the vertebrate MHC (Penn *et al.* 2002). The difference in resistance across ploidies is due to the increased frequency of heterozygous genotypes present in autotetraploid versus diploid populations. When the parental diploid host population is nearly monomorphic (low variance), the tetraploid population does not contain a significantly greater proportion of heterozygous genotypes, and therefore the difference in resistance is minimal. In addition, our results show that the largest differences in resistance occur when host and pathogen allele frequency distributions have similar means. Intuitively, this is because the diploid host population has a moderate level of resistance, allowing for larger differences in resistance between ploidies.

To this point, we have only considered a single genetic locus. Since host resistance to pathogens is frequently determined by multiple epistatically interacting loci (Frank 1994) however, we evaluated whether our results hold when the number of loci is increased. In order to provide a conservative prediction for differences in resistance across ploidies, multiple diallelic loci were incorporated by assuming an extreme form of epistasis in which hosts resistant at a single locus were completely

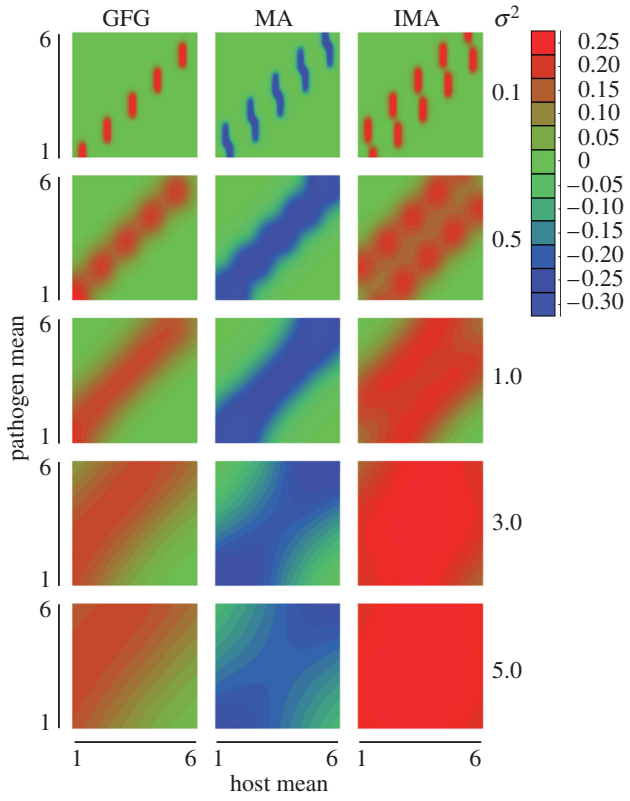

Figure 1. The difference in resistance between tetraploid and diploid hosts as a function of the mean of the allele frequency distributions for host and pathogen. Red represents parameter combinations for which tetraploid resistance is greater, and blue, parameter combinations for which diploid resistance is greater. The distribution of allele frequencies was assumed to be Gaussian in both host and pathogen, with mean and variance (σ^2) as specified in the figure. Maximum differences in resistance for the MA and IMA occur where the host and pathogen allele frequency distributions have similar means and polymorphism (variance) is high. For the GFG, alleles further along the positive horizontal axis represent increasing host resistance and alleles further along the vertical axis represent increasing pathogen virulence. Note that the difference in resistance is sometimes close to zero, but never equal to zero. This figure assumes that six alleles govern the interaction although assuming a different number of alleles yields similar results (electronic supplementary material, figure S6).

resistant to the pathogen (electronic supplementary material). Extending the analyses described above to this case was straightforward for the IMA model. Our results showed that as with the case of a single locus, tetraploids are always more resistant. For the MA and GFG models, however, we were unable to derive simple analytical expressions and so calculated expected levels of resistance numerically (electronic supplementary material). For the GFG and IMA models, as the number of loci involved in pathogen resistance increased, the difference in resistance between the ploidies decreased, although the sign of the difference never changed. This result arises because we have assumed that resistance at any single locus conferred resistance to the host, such that large numbers of loci lead to increased resistance in both diploid and tetraploid host populations, thus reducing the maximum possible difference in resistance that can occur across ploidies. This effect is particularly strong for the IMA model. In contrast, for the MA model, increasing the number of loci increased the difference in resistance due to the host populations becoming generally less resistant. If resistance in natural populations is determined by the multiplicative action of many resistance loci, or by some other more moderate form of epistasis than we have modelled, increasing numbers of loci would have a much less significant effect.

Newly formed autotetraploid populations are frequently small, creating the potential for genetic drift to erode heterozygosity and potentially reduce predicted differences in resistance across ploidy. We evaluated this possibility using individual-based computer simulations (electronic supplementary material). These simulations demonstrated that if novel tetraploid populations are founded by only a single individual, irrespective of whether this individual is annual or perennial, the difference in tetraploid resistance is short lived, and eliminated on average after three generations for all models considered. In contrast, for novel tetraploid populations of perennials founded by multiple individuals, the effect persisted indefinitely, although at a reduced level (electronic supplementary material, figures S4 and S5). In order to isolate the effects of small population size and genetic drift on levels of tetraploid resistance, simulations did not include processes such as mutation and selection. However, these processes could clearly play a role in the long-term evolutionary success of a polyploid lineage. In addition, forces such as gene flow, disassortative mating and mutation, which can bolster heterozygosity would limit the effects of drift and promote a durable increase in tetraploid resistance. Thus we consider these results to be quite conservative.

Although recent work challenges the need to explain the perceived abundance of polyploid lineages (Meyers & Levin 2006), the specific mechanisms that allow neopolyploid populations to establish themselves are still poorly understood as a whole (Ramsey & Schemske 2002). Current hypotheses suggest that selfing, asexuality, perenniality, assortative mating and triploid hybrids may aid in establishment (Ramsey & Schemske 1998; Otto & Whitton 2000; Husband 2004; Husband & Sabara 2004; Rausch *et al.* 2005). Additionally, polyploid lineages tend to vary phenotypically from their diploid progenitors due to novel gene expression (Osborn *et al.* 2003). Typical differences include larger cell and seed size, slower development, altered hormone levels and wider ecological range (Levin 1983; Otto & Whitton 2000). While these phenotypic effects may offer some selective advantage in specific situations, few seem universally beneficial or potent enough to overcome the disadvantage of minority cytotype exclusion. Owing to the ubiquity of host–pathogen interactions, a general increase in pathogen resistance under the models with broadest empirical support (GFG and IMA; Burdon 1987) offers a more palatable explanation for successful polyploid establishment.

Our results are consistent with the general finding that polyploids tend to be perennials (Stebbins 1938, 1971). In the absence of fitness benefits from increased pathogen resistance, our results show that genetic drift can quickly erode the increased heterozygosity driving improved pathogen resistance in initially small populations of polyploid annuals. In contrast, our results show that for perennial species with multiple founders, increased pathogen resistance can be maintained, particularly when polyploids are formed recurrently, as has been

recently demonstrated in a variety of systems (Ness *et al.* 1989; Soltis & Soltis 1999).

Conclusive empirical tests of the theory presented here will require multiple independent comparisons of pathogen resistance in early generation polyploids and their diploid progenitors. Studies that use long established polyploid populations (Thompson *et al.* 1997, 2004; Nuismer & Thompson 2001) cannot adequately test the theory due to the potential for long-term coevolutionary change not incorporated in the current model. The significant variability in the resistance of novel polyploid populations revealed by our results (electronic supplementary material, figure S3) also suggests that empirical studies will need to evaluate the pathogen resistance of many recently and independently formed polyploid individuals.

In addition to providing novel insights into the role interactions with pathogens play in promoting the establishment of polyploid populations, the theory developed here offers a fresh approach to addressing the long-standing debate over the genetic basis of pathogen resistance (Frank 1994; Parker & Gilbert 2004). Specifically, empirical studies of relative levels of pathogen resistance in diploids and synthetically generated autotetraploids could be used to distinguish between the models of pathogen resistance considered here. For instance, if synthetic autotetraploids are consistently found to be more resistant to pathogens than their diploid progenitors, it can be reasonably inferred that resistance is governed by the GFG or IMA model. If, in contrast, empirical studies reveal consistent decreases in autotetraploid resistance, support is provided for the MA model. Such studies, in combination with further theoretical refinements, provide a novel and potentially fruitful path to unravelling the genetic basis of pathogen resistance in plants.

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