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# Outcrossed sex allows a selfish gene to invade yeast populations

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Homing endonuclease genes (HEGs) in eukaryotes are optional genes that have no obvious effect on host phenotype except for causing chromosomes not containing a copy of the gene to be cut, thus causing them to be inherited at a greater than Mendelian rate via gene conversion. These genes are therefore expected to increase in frequency in outcrossed populations, but not in obligately selfed populations. In order to test this idea, we compared the dynamics of the *VDE* HEG in six replicate outcrossed and inbred populations of yeast (*Saccharomyces cerevisiae*). *VDE* increased in frequency from 0.21 to 0.55 in four outcrossed generations, but showed no change in frequency in the inbred populations. The absence of change in the inbred populations indicates that any effect of *VDE* on mitotic replication rates is less than 1%. The data from the outcrossed populations best fit a model in which 82% of individuals are derived from outcrossing and *VDE* is inherited by 74% of the meiotic products from heterozygotes (as compared with 50% for Mendelian genes). These results empirically demonstrate how a host mating system plays a key role in determining the population dynamics of a selfish gene.

**Keywords:** selfish gene; outcrossed sex; population invasion; homing endonuclease; yeast; intein

## 1. INTRODUCTION

Homing endonuclease genes (HEGs) are optional or non-essential genes that are widely distributed within fungi, protists, bacteria and viruses (Mueller *et al*. 1993; Belfort & Roberts 1997). They have no known host function, at least among eukaryotes, and instead are thought to be selfish or parasitic genes that spread in populations because their catalytic activity results in a biased pattern of inheritance (Hickey 1982). Any particular HEG exists only at one site in the genome and codes for an enzyme that specifically recognizes and cleaves sites not containing the gene. Thus, in heterozygous individuals, in which there are both $HEG^+$ and $HEG^-$ sites, the latter are cleaved by an enzyme made by the former. The cell then repairs the cut chromosome in the normal way, which involves using the intact $HEG^+$ chromosome as a template for repair (Szostak *et al*. 1983; Colaiacovo *et al*. 1999). Thus, after repair the heterozygote has been converted into an $HEG^+$ homozygote (figure 1). Consequently, these genes show strong transmission ratio distortion, often being inherited by up to 95% of progeny, rather than the Mendelian 50%.

Phylogenetic surveys show that HEGs are sporadically distributed among closely related species, and sequence analysis indicates that some of these HEGs contain frame-shift mutations and are presumably non-functional (Goddard & Burt 1999). There is also strong phylogenetic evidence for the rampant horizontal transmission of HEGs (Vaughn *et al*. 1995; Goddard & Burt 1999; Koufopanou *et al*. 2002). These observations led to the construction of a cyclical model of HEG evolution which suggests that horizontal transfer events introduce HEGs to uninfected populations, and that this is followed by HEG population invasion, then degeneration and, finally, loss; the cycle may then be initiated once more with another HEG horizontal transfer event (Goddard & Burt 1999). Frequent horizontal transfer may allow HEGs to persist over evolutionary time by the recurrent invasion of new species or populations. One critical assumption of this model is that a newly introduced HEG will indeed spread to fixation. In the absence of any countervailing forces, the transmission ratio distortion shown by an HEG will lead to it increasing in frequency in an outcrossed sexual population. However, this has never been empirically demonstrated and may be prevented if, for example, the gene substantially reduces host fitness. Moreover, all else being equal, HEGs should not increase in frequency in a wholly inbred population since gametes from independent $HEG^+$ and $HEG^-$ lineages are not brought together and provide no opportunity for super-Mendelian inheritance. HEGs may only increase within inbred populations if they confer a benefit or if there is an extremely high rate of horizontal transfer between lineages (an upper bound estimate for the HEG horizontal transfer rate encompasses infinity) (Goddard & Burt 1999; Koufopanou *et al*. 2002). Previous studies in yeast have shown that other types of selfish element, in particular the 2 μm plasmid (Futcher *et al*. 1988) and Ty3 element (Zeyl *et al*. 1996), can indeed increase in frequency in sexually outcrossed populations but not in inbred ones. As yet, there are no similar data concerning HEG population dynamics.

*VDE* is one of the best-studied HEGs and infects the middle of the metabolically important *VMA1* gene (which codes for a subunit of the vacuolar ATP pump) (Gimble & Thorner 1992). Ordinarily, an insertion within *VMA1* should destroy its function. However, the ATP pump subunit derived from $VDE^+$ alleles is not compromised since *VDE* self-splices at the protein level (Chong *et al*. 1996) to leave a functionally intact VMAlp and the free *VDE* protein product PI-*Sce*I; such elements are known as inteins (Colston & Davis 1994). PI-*Sce*I has an endonuclease function: it uniquely recognizes *VMA1* alleles

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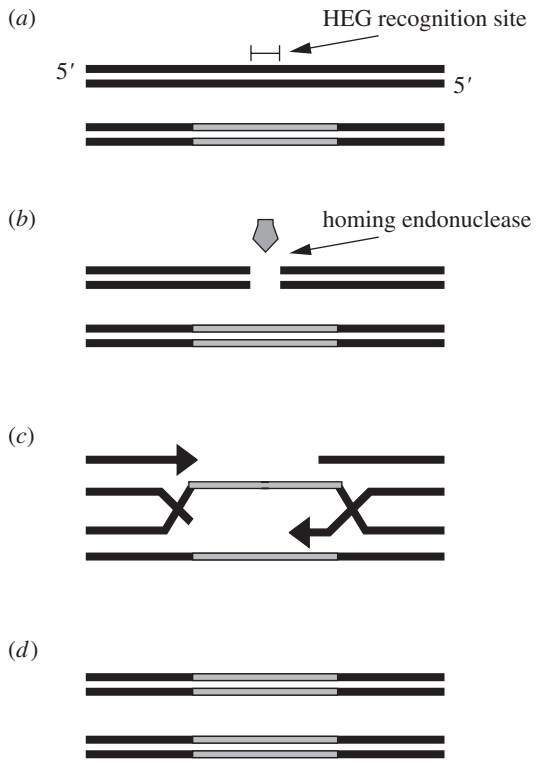


Figure 1. Model of 'homing' via gene conversion with either the double-strand break repair or synthesis-dependent strand annealing pathway (Szostak *et al*. 1983; Colaiacovo *et al*. 1999); light boxes represent an HEG. (*a*) Recipient ($HEG^-$) and donor ($HEG^+$) alleles. (*b*) The endonuclease is transcribed and translated from the $HEG^+$ allele and recognizes and cuts a specific sequence within the $HEG^-$ allele. This sequence is split into two and, therefore, destroyed by the insertion of the HEG. (*c*) Repair of the break using the $HEG^+$ allele as a template. (*d*) Resolution of duplexes.

that do not contain *VDE* and cuts them at the exact point where *VDE* is inserted in $VDE^+$ alleles (Gimble & Wang 1996). This break initiates the cell's repair pathway, which results in the conversion of $VDE^-$ alleles and, thus, facilitates *VDE*'s super-Mendelian inheritance. We test the validity of ideas concerning the HEGs capability for population invasion by comparing *VDE*'s change in frequency between experimentally inbred and outcrossed populations of *Saccharomyces cerevisiae*.

## 2. MATERIAL AND METHODS

### (a) *Strains and microbiological methods*

We constructed isogenic $VDE^+$ and $VDE^-$ strains of *S. cerevisiae* by transforming the haploids DH89 *MAT*α *ho ura3* and DH90 *MAT***a** *ho ura3* (which are descendants of the wild-type Y55) with the YEpVMA1 plasmid (Gimble & Thorner 1993), which contains the $VDE^+$ allele. These haploids were mated and put through meiosis, which allowed *VDE* to home into the genomic $VDE^-$ allele; the resulting $VDE^+$ strains DH91 and 95 were subsequently cured of the plasmid. The two pairs of haploids were then mated in order to form homozygous diploids (DH89/90 and DH 91/95). These were used for founding six populations that contained the $VDE^+$ allele at an average frequency of 0.21. Each population was divided into two in order to initiate replicate inbred and outcrossed lines (see table 1). The populations were then starved of nitrogen by placing them on 2% potassium acetate and were thus stimulated to go through meiosis and produce four haploid spores (Burke *et al*. 2000). Yeast spores may be one of two mating types, either **a** or α and gametes will only mate with an opposite mating type (though haploids may divide mitotically if no opposite mating type is encountered) (Burke *et al*. 2000). These four yeast spores are contained within an ascus (sac) and, under normal conditions, will germinate when placed on YPD (1% yeast extract, 2% peptone and 2% glucose), mate with their ascus partners and, therefore, inbreed. In order to facilitate outcrossing asci were broken apart by overnight digestion with 4.4 mg $ml^{-1}$ sulphatase (Sigma no. S9626) at 30 °C and then mildly sonicated in order to dissociate the asci and randomize the spores fully. The spores were allowed to mate randomly and then grow by placing them on YPD for *ca*. 15 h at 30 °C (this equates to a maximum of 10 mitotic generations) before the next round of sporulation. The cells were removed from the YPD and washed with water before again placing them on 2% potassium acetate. Both inbred and outcrossed replicate populations simultaneously passed through five sexual (meiotic) generations. Since all individuals were homozygous at the start, the opportunity for *VDE* to display super-Mendelian inheritance only arose in generations 2–5 once heterozygous genotypes were produced.

For the purposes of comparison we also measured the frequency of *VDE* in the meiotic products arising from $VDE^+/VDE^-$ heterozygotes. Spores from 24 tetrads were dissected, allowed to form haploid colonies and then scored for the presence/absence of *VDE*.

### (b) *Molecular methods*

The frequency of *VDE* was determined at generations 0, 3 and 5 by a colony polymerase chain reaction (PCR). Samples from each population were plated at low density on YPD and 95 colonies were randomly selected. The ploidy of these samples was determined by transferring them to sporulation medium, waiting 5 days, exposing them to ether vapours (Rockmill *et al*. 1991) and then replica plating to YPD. Only diploids would be able to make spores that could survive the ether treatment. Less than 1% of the sample colonies did not sporulate; these were presumably unmated haploids (table 1). Original sample colonies were then suspended in 1 × PCR buffer, incubated at 98 °C for 5 min and then vortexed and centrifuged at 13 000 rpm for 2 min. Ten microlitres of supernatant was used as a template in a PCR reaction with the primers VMA105 (5′-CAAGTACTC CAATTCTGAC) and VMA102 (5′-ATTCCATCAAGACTTC TGC), which flank the *VDE* insertion site. The sizes of the PCR products indicated the *VDE* status of each yeast colony: a small amplicon indicated a $VDE^-$ allele (80 bp), a large amplicon indicated a $VDE^+$ allele (1448 bp) and two amplicons indicated a heterozygote. The PCR products were electrophoresed through 1.5% agarose in order to determine their size. The 95 PCR reactions for each sample point were performed using a 96-well plate, with the 96th well containing a known $VDE^+/VDE^-$ heterozygote as a positive control. Since *VDE* only homes during meiosis and not during mitosis (Gimble & Thorner 1993), we used the frequency of $VDE^+/VDE^-$ heterozygotes for estimating the outcrossing efficiency.

## 3. RESULTS

The extent of inbreeding experienced by each of the populations was determined by calculating Wright's

Table 1. Frequencies of *VDE* homozygous (+/+), heterozygous (+/−) and *VDE*-free (−/−) genotypes in each of the replicate inbred and outcrossed populations estimated from the 95 individuals sampled from each population at each time-point using the colony PCR method.

(The number of individuals disregarded because they were determined to be haploid are indicated in the columns headed ln, the estimated frequency of *VDE* is shown for each population at each time-point in the columns headed *p* and inbreeding coefficients are shown in the columns headed *F*.)

| | meiotic generation | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | | | | | | 3 | | | | | | 5 | | | | | |
| | population | ln | +/+ | +/− | −/− | *p* | population | ln | +/+ | +/− | −/− | *p* | *F* | ln | +/+ | +/− | −/− | *p* | *F* |
| | | | | | | | A | 0 | 21 | 0 | 74 | 0.22 | 1.00 | 0 | 21 | 0 | 74 | 0.22 | 1.00 |
| | | | | | | | B | 0 | 20 | 0 | 75 | 0.21 | 1.00 | 1 | 23 | 0 | 71 | 0.24 | 1.00 |
| | | | | | inbred | | C | 0 | 18 | 0 | 77 | 0.19 | 1.00 | 0 | 20 | 0 | 75 | 0.21 | 1.00 |
| | | | | | | | D | 0 | 20 | 0 | 75 | 0.21 | 1.00 | 2 | 22 | 0 | 71 | 0.24 | 1.00 |
| | A | 0 | 22 | 0 | 73 | 0.23 | E | 0 | 21 | 0 | 74 | 0.18 | 1.00 | 1 | 19 | 0 | 75 | 0.20 | 1.00 |
| | B | 0 | 19 | 0 | 76 | 0.20 | F | 0 | 22 | 0 | 73 | 0.23 | 1.00 | 0 | 21 | 0 | 74 | 0.22 | 1.00 |
| | C | 0 | 20 | 0 | 75 | 0.21 | | | | | | | | | | | | | |
| | D | 0 | 22 | 0 | 73 | 0.23 | | | | | | | | | | | | | |
| | E | 0 | 20 | 0 | 75 | 0.21 | A | 2 | 12 | 38 | 43 | 0.33 | 0.08 | 0 | 28 | 41 | 26 | 0.51 | 0.14 |
| | F | 0 | 19 | 0 | 76 | 0.20 | B | 0 | 15 | 34 | 46 | 0.34 | 0.20 | 0 | 30 | 40 | 25 | 0.53 | 0.16 |
| | | | | | outcrossed | | C | 0 | 14 | 39 | 42 | 0.35 | 0.10 | 0 | 27 | 39 | 29 | 0.49 | 0.18 |
| | | | | | | | D | 1 | 17 | 39 | 38 | 0.39 | 0.13 | 2 | 31 | 42 | 20 | 0.56 | 0.08 |
| | | | | | | | E | 1 | 16 | 36 | 42 | 0.36 | 0.17 | 2 | 34 | 46 | 13 | 0.61 | −0.04 |
| | | | | | | | F | 0 | 20 | 45 | 30 | 0.45 | 0.04 | 0 | 39 | 41 | 15 | 0.63 | 0.08 |

inbreeding coefficient (*F*) (table 1), which estimates the deviation of a population from Hardy–Weinberg equilibrium (Weir 1996). The mean (± s.e.) inbreeding coefficients for the outcrossed and inbred populations were $0.11 \pm 0.019$ and $1.0 \pm 0.0$, respectively, demonstrating that asci digestion and spore randomization were effective at producing largely outcrossed populations. The frequency of *VDE* increased significantly in the outcrossed populations, but not in the inbred populations (paired *t*-tests of arcsine-transformed initial and final frequencies $p < 0.001$ and $p = 0.34$, respectively) (figure 2). This dependency of gene frequency change on the breeding system indicates that the increase is a result of super-Mendelian inheritance and not selection for cells that are $VDE^+$.

If we assume that the inbred populations are completely inbred (and the failure to detect even a single heterozygote suggests this is not too far wrong), then the change in frequency of *VDE* in these populations can be used for estimating the selection coefficient associated with *VDE*. The fact that the initial and final frequencies did not differ significantly means the selection coefficient is not significantly different from zero. In order to put bounds on the selection coefficient, we calculated the regression of $\ln(p/1-p)$ on the number of mitotic generations, assuming 10 mitotic generations per meiotic generation. The mean (± s.e.) regression coefficient across the six replicate populations is $0.0009 \pm 0.00099$. Our value of 10 mitotic generations per meiotic generation is an upper limit; if, instead, we assume that there were five mitotic generations, then the selection coefficient is $0.002 \pm 0.0020$. It appears that any effect *VDE* may have on cell replication rates, either positive or negative, is small (< 1%) (at least in our experimental environment). *VDE*'s capability for population invasion will therefore depend principally on the rate of inheritance in heterozygotes and on the frequency of outcrossing.

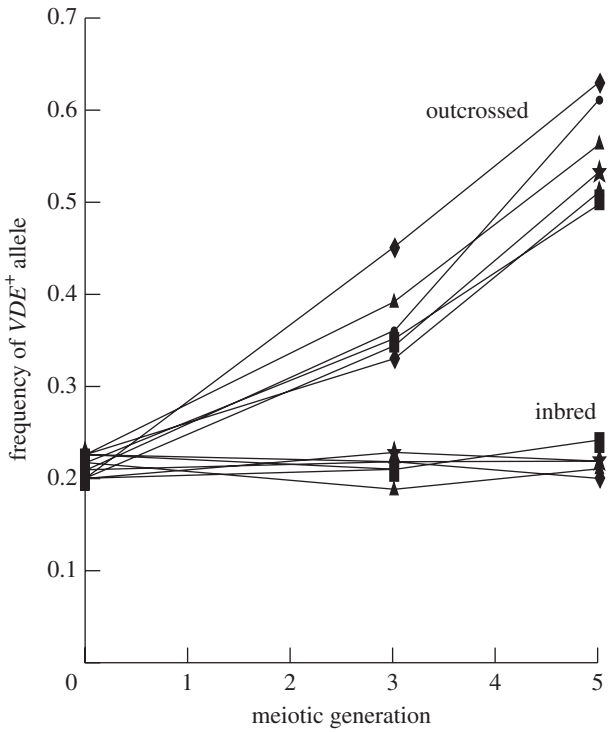


Figure 2. The change in frequency of *VDE* within replicate inbred and outcrossed experimental yeast populations. Each symbol represents a replicated inbred and outcrossed population.

In order to analyse the spread of *VDE* through the outcrossed populations in more detail, we constructed a model of a selectively neutral super-Mendelian gene in a population with a mixed mating system in which a

fraction $t$ of zygotes are produced by outcrossing and a fraction $1-t$ of zygotes are produced by intra-ascus selfing (see Appendix A). If the frequencies of $VDE^+/VDE^+$, $VDE^+/VDE^-$ and $VDE^-/VDE^-$ individuals in one generation are $x$, $y$ and $z$, then their frequencies in the next generation will be

$$x' = t(x+dy)^2 + (1-t)[x + (1/3)(2d(d+1)-1)y], \quad (3.1)$$

$$y' = 2t[1-(x+dy)](x+dy) + (2/3)(1-t)(1-d)(2d+1)y \quad (3.2)$$

and

$$z' = t(x+dy-1)^2 + (1-t)[(2/3)(d-1)^2 y + z], \quad (3.3)$$

where $d$ is the frequency of *VDE* in the meiotic products of $VDE^+/VDE^-$ heterozygotes ($d=0.5$ being Mendelian inheritance). This model accounts for the fact that intra-ascus mating leads to a reduction in heterozygosity of one-third every generation; this differs from cases in which selfing involves the fusion of gametes from independent meiosis, where heterozygosity is decreased by one-half every generation (Falconer 1981). We used this model for calculating best estimates for $d$ and $t$ by maximizing the likelihood of obtaining the observed data assuming a multinomial distribution (Edwards 1972). The maximum likelihood estimates were $d=74\%$ (95% CI = 72.1–75.3%) and $t=82\%$ (95% CI = 78.2–87.3%) (with the 95% confidence intervals being obtained from bootstrap datasets). *VDE*'s rate of inheritance was also estimated independently by sporulating a heterozygous strain and genotyping the spores directly. Out of 96 spores, 76 were $VDE^+$, giving $d=79\%$ (95% CI = 70–86%, as calculated using the binomial distribution from Rohlf & Sokal (1995)). This alternative estimate agrees very well with the value of $d$ from the experimental populations.

## 4. DISCUSSION

Deviations from Mendelian inheritance can only affect population gene frequencies to the extent to which the population contains heterozygotes. The frequency of heterozygotes is greater with outcrossing than with inbreeding. Therefore, one can assess the role of super-Mendelian inheritance in gene frequency changes by comparing those changes in inbred and outcrossed populations. Futcher *et al*. (1988) were the first to use this approach in their study of the yeast 2 μm plasmid. The breeding system was manipulated in much the same way as was done here, though they had no independent means of estimating the actual outcrossing rates under the two treatments. The plasmid increased in frequency from 0.1 to 0.4 in four sexual generations in two replicate outcrossed populations, whereas there was no change in frequency in the inbred populations. They concluded that the increase in frequency in the outcrossed populations was due to super-Mendelian inheritance, consistent with the plasmid's behaviour in defined crosses. They estimated a cost of the plasmid for mitotic replication rates of *ca*. 1% in separate experiments. We also observed an increase in frequency in outcrossed populations but not inbred populations in our experiments on *VDE* and conclude that the increase in the outcrossed populations is due to super-Mendelian inheritance. Our experiments were of sufficient size that the absence of a change in the inbred populations indicates a small effect (positive or negative) of *VDE* on the mitotic replication rates, certainly less than 1%. These results also show that horizontal transmission of *VDE* is not so rampant as to have a detectable effect on population dynamics in the laboratory.

This logic of outcrossed sexuality facilitating the spread of super-Mendelian elements is also thought to underlie certain facts about the comparative distribution of selfish genetic elements, in particular that B chromosomes are more common in outcrossed species of plants than in inbred species (Burt & Trivers 1998) and that retrotransposable elements are present in most animals except bdelloid rotifers, which are putatively anciently asexual (Arkhipova & Meselson 2000).

The strength of super-Mendelian inheritance in favour of a gene can be quantified by $d$, the fraction of haploid meiotic products produced by heterozygotes that carry the gene. The best estimate from our experimental populations was $d=74\%$, which is similar to the value derived from dissecting tetrads (79%). These values are slightly lower than the 90% (95% CI = 83–95%) reported by Gimble & Thorner (1992), which was derived from dissecting tetrads. This difference may be due to genetic background effects, as the strains used in the two studies were different. Nevertheless, even the lower values are such that *VDE* can dramatically increase in frequency over evolutionarily trivial time-scales. In our experimental populations *VDE* increased from 21 to 56% in just four outcrossed generations, and in a fully outcrossed population it is predicted to increase in frequency from 0.1 to 99.9% in just 29 generations (as calculated by iterating equations (3.1)–(3.3)).

These predictions apply to a fully outcrossed population and, to the extent that natural yeast populations are inbred, these times will increase. Molecular analysis of a natural population of *Saccharomyces paradoxus* (an undomesticated close relative of *S. cerevisiae*) shows strong deviations from Hardy–Weinberg proportions, with an inbreeding coefficient of $F=0.99$ (L. Johnson, personal communication). This high level of inbreeding will retard the spread of a selectively neutral super-Mendelian gene 100-fold. It will also lower the maximum cost that a selfish gene can impose on its host and still spread through a population. *Saccharomyces cerevisiae* is host to a diverse community of selfish genetic elements: in addition to *VDE* and the 2 μm plasmid, there are also seven mitochondrial HEGs (and five more group I introns that probably once had them) (Lambowitz & Belfort 1993), two retrohoming group II introns (Bonen & Vogel 2001), five retrotransposable element families, four RNA viruses and associated satellites (which, despite the name, are vertically inherited and not infectious) (Wickner 1992) and two self-propagating prion protein conformations (Wickner *et al*. 1996). Notably absent from this list are DNA transposons and long interspersed nuclear elements (LINE)-like retrotransposons. Perhaps these are too costly to invade such highly inbred populations.

The high frequencies of inbreeding in *S. paradoxus* are presumably due both to intra-ascus mating and to mating-type switching, which is a molecular mechanism that allows cells to mate with genetically identical clone

mates (Takahashi *et al*. 1958). Mating-type switching proceeds by a directed gene conversion event and the key gene involved (*HO*) encodes a site-specific endonuclease derived from *VDE* (Dalgaard *et al*. 1997). It seems that increased inbreeding has evolved by the domestication of an HEG, an event that, perversely, makes the spread of HEGs and other selfish genes more difficult.

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## APPENDIX A

### (a) *Dynamics of a super-Mendelian gene in a yeast population with a mixed mating system*

We want to calculate the frequencies of $VDE^+/VDE^+$, $VDE^+/VDE^-$ and $VDE^-/VDE^-$ individuals in one generation, given that their frequencies in the previous generation were $x$, $y$ and $z$. Recall that $VDE^-$ alleles are only converted to $VDE^+$ alleles during meiosis. First consider the individuals derived from random mating. The frequency of *VDE* in the gametes will be $u=x+yd$, where $d$ is the frequency of *VDE* in the meiotic products of $VDE^+/VDE^-$ heterozygotes ($d=0.5$ being Mendelian inheritance). The frequencies of the three genotypes among outcrossed zygotes will then be $u^2$, $2u(1-u)$ and $(1-u)^2$, respectively. Now consider the individuals derived from intra-ascus mating. Tetrads derived from homozygous parents will give rise to homozygous offspring. Tetrads derived from heterozygous parents will have two, three or four haploid spores that are $VDE^+$, with the remainder being $VDE^-$. Assuming independent conversion of the two $VDE^-$ alleles, the relative frequencies of these three tetrad types will be $(1-c)^2$, $2c(1-c)$ and $c^2$, respectively, where $c=2d-1$ is the probability that a particular $VDE^-$ allele is converted to a $VDE^+$ allele ($c=0$ being Mendelian inheritance). The relative frequencies of the three diploid genotypes are then calculated by assuming the meiotic products fuse randomly with respect to *VDE* status (note the *VDE* locus is unlinked to the mating type locus). Overall, the frequencies of the three genotypes will be

$$x' = tu^2 + (1-t)\{x + y[(1-c)^2(1/6) + 2c(1-c)(1/2) + c^2]\}, \quad \text{(A 1)}$$

$$y' = 2tu(1-u) + (1-t)y[(1-c)^2(2/3) + 2c(1-c)(1/2)] \quad \text{(A 2)}$$

and

$$z' = t(1-u)^2 + (1-t)\{z + y[(1-c)^2(1/6)]\}, \quad \text{(A 3)}$$

which simplify to equations (3.1)–(3.3).

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