Introduction
Facultative sex is widespread in nature and can be seen in a wide variety of animal, plant and fungal species (e.g. Daphnia, aphids, cacti and Aspergillus). These species often tend to reproduce sexually when stressed. However, it is not known if the preference for sexual reproduction is because of plasticity due to a poor environment or because of condition-dependent sex (Figure 1). It has been theorized that high-fitness genotypes would prefer to use asexual reproduction since a large number of high-fitness offspring will be produced. Low-fitness genotypes would prefer to use sexual reproduction in order for the chance to obtain a beneficial mutation resulting in better genes. The filamentous fungus, Aspergillus nidulans, can have either an asexual or sexual reproductive cycle (Figure 2). Previous research has shown that selfing was higher in genotypes with lower fitness (i.e. condition-dependent selfing, Schoustra et al, 2010). This experiment aims to determine if outcrossing is also condition-dependent.

Methodology
The fitness proxy used to measure the fitness of each of 32 A. nidulans strains was the proportion of nuclei of the focal strain relative to those of a tester strain. Two measures of the investment in sexual reproduction were used: the average proportion of selfed to outcrossed cleistothecia (out of 50 cleistothecia examined) and the average proportion of selfed to outcrossed ascospores (out of 50 cleistothecia). Genetic markers associated with spore colour (yellow, green, and white) were used to examine the offspring of a mating between a tester strain and each of the 32 wild type focal strains (Figure 3). The offspring from each cross was grown on complete medium (Schoustra et al, 2010). The proportion of outcrossed offspring was measured based on the number of offspring showing evidence of segregation of the two mutant alleles (expectation of 25% yellow offspring in outcrossed cleistothecia).

Condition-dependent sex
Figure 1. Model of the research question. Plasticity is the ability of a genotype to change its phenotype in response to its environment. Condition-dependent sex is the environment affecting the species’ fitness which in turn dictates whether reproduction will be sexual or asexual.

Figure 2. Reproductive cycles of A. nidulans. Aspergillus asexual cycle begins with the formation of conidiphores followed by the release of conidiospores. The sexual cycle begins with the fusion of two haploid cells to form a diploid cell. The two haploid cells may come from the same individual (selfing) or from different individuals (outcrossing). The diploid cell then undergoes meiosis and cleistothecia (sexual fruiting bodies) containing ascospores are produced.

Results
Figure 3. Expected results of focal and tester cross. A focal strain with wild type alleles ( ) coding for the production of green spores was crossed with a tester with mutant alleles ( † ) coding for the production of yellow (y) or white (w) spores. The white allele is epistatic to the yellow allele (i.e. the tester produces white spores). Based on Mendelian segregation, The expected phenotypic results based on Mendelian segregation, 25% green, 25% yellow and 50% white spores are expected.

Figure 4. The equation for the linear model is Proportion of outcrossed ascospores = 0.50 × Relative frequency of nuclei from the focal strain + 0.52. The P-value is 0.00805. The R² value is 0.08662. This supports the hypothesis: the lower the fitness (lower relative frequency of the focal strain) the higher proportion of outcrossed ascospores.

Figure 5. The equation for the linear model is Proportion of outcrossed cleistothecia = 0.056 × Relative frequency of nuclei from the focal strain + 0.37. The P-value is 0.7687. The R² value is 0.001678.

Conclusion
The P-value was significant (<0.05) for the linear model of the proportion of outcrossed ascospores compared to the relative frequency of focal strain nuclei. This means that the hypothesis was confirmed. A higher proportion of outcrossed ascospores was observed when the strain had a lower fitness. The P-value for the proportion of outcrossed cleistothecia was not significant. So no definite conclusions can be made. However, a negative correlation was observed. The reason that we do not see a significant relationship in the proportion of outcrossed cleistothecia could be because the number of ascospores in each cleistothecium is not the same. The proportion of outcrossed ascospores is a better measures of investment in sexual reproduction since ascospores are closer to the end of the reproductive cycle.

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